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# Water Quality Renovation of Animal Waste Lagoons Utilizing Aquatic Plants

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# Water Quality Renovation of Animal Waste Lagoons Utilizing Aquatic Plants

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16. ABSTRACT

Duckweeds Spirodela oligorhiza, S. polyrhiza, and Lemna gibba (clone G3) grown on dairy waste lagoons gave an estimated maximum annual yield of 22,023 kg dry wt./ha. S. oligorhiza and L. gibba had higher growth rates in the spring, fall, and winter, with L. gibba growing throughout most of the winter. Nutrient content of the plants increased with increasing nutrients in the lagoons. Mean crude protein of dry duckweeds was 36%, to a maximum of 42%. Maximum protein yield/$m^2$ exceeded protein produced by peanuts, soybeans, and cottonseed 9, 12, and 40 fold respectively. The duckweeds recovered on a hectare basis the N, P, and K of 15.5, 34, and 8.8 lactating cows respectively. Reductions in lagoon TKN, $NH_4^+$, and P were significantly greater in the duckweed lagoons than controls. Reduction of TKN averaged 0.91 mg/l/day in summer for duckweed-covered lagoons and 0.74 mg/l/day for controls. During the winter the rate was 1.27 mg/l/day (duckweed lagoons) and 0.82 mg/l/day for controls. Ammonium reduction was 84% greater in the duckweed lagoons during winter. Phosphorus reduction in duckweed lagoons, though significantly different from controls, was insufficient to meet water quality standards.

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# WATER QUALITY RENOVATION OF ANIMAL WASTE LAGOONS UTILIZING AQUATIC PLANTS

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This report has been reviewed by the Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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# FOREWORD

The Environmental Protection Agency was established to coordinate administration of the major Federal programs designed to protect the quality of our environment.

An important part of the Agency's effort involves the search for information about environmental problems, management techniques and new technologies through which optimum use of the nation's land and water resources can be assured and the threat pollution poses tc the welfare of the American people can be minimized.

EPA's Office of Research and Development conducts this search through a nationwide network of research facilities.

As one of these facilities, the Robert S. Kerr Environmental Research Laboratory is responsible for the management of programs to: (a) investigate the nature, transport, fate and management of pollutants in groundwater; (b) develop and demonstrate methods for treating wastewaters with soil and other natural systems; (c) develop and demonstrate pollution control technologies for irrigation return flows, (d) develop and demonstrate pollution control technologies for animal production wastes; (e) develop and demonstrate technologies to prevent, control or abate pollution from the petroleum refining and petrochemical industries, and (f) develop and demonstrate technologies to manage pollution resulting from combinations of industrial wastewaters or industrial/municipal wastewaters.

This report is a contribution to the Agency's overall effort in fulfilling its mission to improve and protect the nation's environment for the benefit of the American public.

William C. Galegar

William C. Galegar, Director
Robert S. Kerr Environmental
Research Laboratory

# ABSTRACT

Duckweeds, Lemnaceae, were grown on a two stage dairy waste lagoon system. *Spirodela oligorhiza* exhibited greater growth than *S. polyrhiza* in spring and early fall, while the opposite occurred during the summer. *Lemna gibba* (Clone G3) grew best during the fall, winter, and early spring. Mixed cultures performed equally well compared to single clone cultures, under static and mixing conditions.

Estimated annual yield on a per hectare basis indicated 22,023 kg (dry weight) theoretically could have been produced based on demonstrated highest treatment yields for each three month period. However, this production is not as high as laboratory studies.

Nutrient content of duckweeds increases when grown on water containing high nutrient concentrations. Mean crude protein content of duckweeds (irrespective of species and treatment) was 36%, but a high of 42% was recorded. Duckweeds compare favorably with ingredients of high protein supplements used in livestock rations. Results indicate that duckweeds may be economical to use as a replacement for some common protein supplement ingredients used in dairy cattle rations.

Based on the estimated annual yield and values of nutrients excreted by dairy cattle, duckweeds recovered the nitrogen of 15.5 head, phosphorus of 34.0 head, and potassium of 8.8 head on a per hectare basis.

Reductions in Total Kjeldahl Nitrogen, ammonium, and phosphorus were significantly greater ($P < .05$) in duckweed covered test channels than in controls. Summer reduction rates of Total Kjeldahl Nitrogen were 0.91 mg/l/day for test channels supporting stands of *S. oligorhiza* and 0.74 mg/l/day for controls. Total Kjeldahl Nitrogen declined during the winter under aerated conditions at rates of 1.27 mg/l/day and 0.82 mg/l/day on *S. oligorhiza* covered channels and controls respectively. Ammonium decline under aerated winter conditions showed rates of 0.035 $mg/l/t^3$ and 0.019 $mg/l/t^3$ for *S. oligorhiza* and controls respectively.

This report was submitted in fulfillment of Grant No. R803326 to Louisiana State University and the Louisiana Agricultural Experiment Station under the partial sponsorship of the U. S. Environmental Protection Agency. This report covers the period July 1, 1975 to December 31, 1977, and the work was completed as of December 28, 1977.

## CONTENTS

Foreword . . . . . . . . . . . . . . . . . . . . . . . . . . . . iii
Abstract . . . . . . . . . . . . . . . . . . . . . . . . . . . . iv
Figures . . . . . . . . . . . . . . . . . . . . . . . . . . . . vi
Tables . . . . . . . . . . . . . . . . . . . . . . . . . . . . viii
List of Metric Conversions . . . . . . . . . . . . . . . . . . xv
Acknowledgements . . . . . . . . . . . . . . . . . . . . . . . xvi

1. Introduction . . . . . . . . . . . . . . . . . . . . . . . 1
2. Conclusions . . . . . . . . . . . . . . . . . . . . . . . 3
3. Recommendations . . . . . . . . . . . . . . . . . . . . . 5
4. Technical Background . . . . . . . . . . . . . . . . . . 7
5. Objectives . . . . . . . . . . . . . . . . . . . . . . . 15
6. Methods . . . . . . . . . . . . . . . . . . . . . . . . . 16
7. Results . . . . . . . . . . . . . . . . . . . . . . . . . 20
    Plant Screening . . . . . . . . . . . . . . . . . . 20
    Waste Treatment Studies . . . . . . . . . . . . . . 20
    Preliminary Feeding Trials . . . . . . . . . . . . . 125
8. Discussion . . . . . . . . . . . . . . . . . . . . . . . 126
9. References . . . . . . . . . . . . . . . . . . . . . . . 137
10. Bibliography . . . . . . . . . . . . . . . . . . . . . . 141

# FIGURES

Number Page

1 Campus dairy-herd facility showing location of feedlot and milking parlor . . . . . . . . . . . . . . . . . . . . . . . . 17

2 Biochemical oxygen demand, chemical oxygen demand, total residue, volatile residue, and fixed residue in static dairy waste lagoon water with and without duckweed (_S. oligorhiza_), spring, 1976 (Test lagoon 1) . . . . . . . . . 31

3 TKN reduction and pH in static dairy waste lagoons with and without stands of duckweeds, spring, 1976 (Test lagoon 1) . . . . . . . . . . . . . . . . . . . . . . . . . 33

4 5-day biochemical oxygen demand in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 48

5 Chemical oxygen demand in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . 49

6 Total residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . . . . . 50

7 Volatile residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . . . . . 51

8 Fixed residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . . . . . 52

9 Total suspended matter in static dairy waste lagoons with and without stands of duckweeds, summer, 1976 . . . . . . 53

10 TKN reduction in test channels covered with duckweed, and controls, summer, 1976 . . . . . . . . . . . . . . . . . . 54

11 TOC reduction in test channels covered with duckweed, and controls, summer, 1976 . . . . . . . . . . . . . . . . . . 55

12 Standard bacteria plate count and anaerobic counts per ml of composite samples of the test channels covered with duckweed, and controls, summer, 1976 . . . . . . . . . . 60

Number Page

13 Fecal coliform per ml of composite samples of test channels covered with duckweed, and controls, summer, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . 61

14 Fecal streptococci count per ml of composite samples of test channels covered with duckweed, and controls, summer, 1976 . . . . . . . . . . . . . . . . . . . 62

15 A schematic plan of an aquacultural waste treatment system involving duckweed . . . . . . . . . . . . . . . . . . . 135

## TABLES

| Number | | Page |
|---|---|---|
| 1 | NUMBER OF ANIMALS MAINTAINED UNDER FEEDLOT CONDITIONS | 8 |
| 2 | CHEMICAL COMPOSITION OF PARTIALLY DRIED *Ricciocarpus natans* AND *Azolla caroliniana* GROWN ON DAIRY WASTE LAGOONS UNDER WINTER CONDITIONS, JANUARY, 1976 | 21 |
| 3 | CHEMICAL COMPOSITION OF DUCKWEED MAINTAINED IN LOW NUTRIENT PONDS BEFORE USE IN LAGOONS RECEIVING DAIRY CATTLE WASTE | 22 |
| 4 | CHEMICAL ANALYSIS OF CITY WATER, LAGOON SOILS, AND MANURE | 23 |
| 5 | BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE OF STAGE 1 ANAEROBIC DAIRY LAGOON EFFLUENT AFTER LOADING BEGAN (FEBRUARY 19, 1976) | 25 |
| 6 | STAGE 1 ANAEROBIC LAGOON TKN AND pH VALUES | 26 |
| 7 | STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE STAGE 1 ANAEROBIC LAGOON EFFLUENT | 27 |
| 8 | RAINFALL DATA FOR TEST 1 STUDY PERIOD (CM) | 29 |
| 9 | BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE IN mg/l OF STATIC DAIRY WASTE LAGOONS WITH AND WITHOUT DUCKWEED (*S. oligorhiza*), SPRING, 1976 (TEST LAGOON 1) | 30 |
| 10 | STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS IN STATIC DAIRY WASTE LAGOONS WITH AND WITHOUT DUCKWEED (*S. oligorhiza*), SPRING, 1976 (TEST LAGOON 1) | 32 |
| 11 | *S. oligorhiza* BIOMASS HARVESTED FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SPRING, 1976 | 34 |

Number Page

12 PARTIAL CHEMICAL ANALYSIS OF S. oligorhiza GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SPRING, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 35

13 PARTIAL CHEMICAL ANALYSIS OF S. polyrhiza GROWN ON TEST CHANNELS RECEIVING A CONTINUOUS FLOW OF DAIRY WASTE FROM THE STAGE 1 ANAEROBIC LAGOON, SPRING, 1976 . . . . . 37

14 BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE OF THE STAGE 1 ANAEROBIC DAIRY LAGOON EFFLUENT . . . . . . . . 39

15 STAGE 1 ANAEROBIC LAGOON WATER QUALITY . . . . . . . . . . . . . . 40

16 STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE STAGE 1 ANAEROBIC LAGOON EFFLUENT . . . . . . . . . . . . . . . . . . . . . . . 41

17 RAINFALL DATA FOR TEST 2 STUDY PERIOD (CM) . . . . . . . . . . . 42

18 BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF THE S. oligorhiza TEST CHANNELS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . 43

19 BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF COMPOSITE SAMPLE OF THE S. polyrhiza TEST CHANNELS, SUMMER, 1976 . . . . . . . . 44

20 BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF THE COMPOSITE SAMPLE FROM THE CONTROL CHANNELS, SUMMER, 1976 . . . . . . . . . . . . 45

21 TKN, POTASSIUM, CALCIUM, TOC, pH AND WATER TEMPERATURE OF TEST AND CONTROL CHANNELS, SUMMER, 1976 . . . . . . . . . . 46

22 STANDARD PLATE COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . 56

23 ANAEROBIC COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . 57

24 FECAL COLIFORM COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . 58

Number Page

25 FECAL STREPTOCOCCI COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . 59

26 PERCENT REDUCTION IN TEST AND CONTROL CHANNEL WATER QUALITY AND BACTERIAL COUNTS, SUMMER, 1976 . . . . . . . . . . . 63

27 _S_. _oligorhiza_ BIOMASS HARVESTED FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . . . 65

28 _S_. _polyrhiza_ BIOMASS HARVESTED FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . . . 66

29 PARTIAL CHEMICAL ANALYSIS OF _S_. _oligorhiza_ GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 67

30 PARTIAL CHEMICAL ANALYSIS OF _S_. _polyrhiza_ GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 68

31 WATER QUALITY OF TEST AND CONTROL CHANNELS UNDER MIXED AND NON-MIXED CONDITIONS . . . . . . . . . . . . . . . . 70

32 CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF MIXED AND NON-MIXED CHANNELS . . . . . . . . . . . . . . . . . . . . . . . 73

33 BIOMASS HARVEST OF _S_. _oligorhiza_ AND _S_. _polyrhiza_ GROWN ON MIXED AND NON-MIXED TEST CHANNELS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 74

34 CHEMICAL COMPOSITION OF _S_. _oligorhiza_ AND _S_. _polyrhiza_ GROWN ON MIXED AND NON-MIXED TEST CHANNELS, SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . . . . . 75

35 WATER QUALITY OF TEST AND CONTROL CHANNELS WITH AND WITHOUT THE ADDITION OF DAIRY WASTE EFFLUENT . . . . . . . . . 77

36 CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 1 RECEIVING DAIRY WASTE EFFLUENT . . . . . . . . . . . . . . . 79

37 CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 2 HELD STATIC . . . . . . . . . . . . . . . . . . . . . . . . 80

Number Page

38 STANDARD PLATE COUNT (SPC), ANAEROBIC COUNT(AC), FECAL COLIFORM (FC), AND FECAL STREPTOCOCCI COUNTS (FSC) OF TEST LAGOON 1 RECEIVING DAIRY WASTE EFFLUENT . . . . . . . . 81

39 STANDARD PLATE COUNT (SPC), ANAEROBIC COUNT (AC), FECAL COLIFORM (FC), AND FECAL STREPTOCOCCI COUNTS (FSC) OF TEST LAGOON 2 HELD STATIC . . . . . . . . . . . . . . . . . . 82

40 *S. oligorhiza* BIOMASS HARVESTED FROM FLOODED AND STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . 83

41 *S. polyrhiza* BIOMASS HARVESTED FROM FLOODED AND STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976 . . . . . . . . 84

42 CHEMICAL COMPOSITION OF *S. oligorhiza* GROWN ON FLOODED AND STATIC TEST CHANNELS, SUMMER, 1976 . . . . . . . . . . . . . . . 85

43 CHEMICAL COMPOSITION OF *S. polyrhiza* GROWN ON FLOODED AND STATIC TEST CHANNELS, SUMMER, 1976 . . . . . . . . . . . . . . . 86

44 WATER QUALITY OF TEST LAGOON 2 RECEIVING DAIRY WASTE EFFLUENT INTERMITTENTLY AND SUPPORTING A STAND OF *S. polyrhiza* AND A MIXED STAND OF *S. polyrhiza* AND S. oligorhiza, LATE SUMMER, 1976 . . . . . . . . . . . . . . . . 88

45 CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 2, FLOODED INTERMITTENTLY WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976 . . . . . . . . . . . . . . . . . . . . . . . 89

46 BIOMASS OF *S. polyrhiza* AND A MIXED CULTURE OF *S. polyrhiza* AND *S. oligorhiza* HARVESTED FROM TEST LAGOON 2 FLOODED INTERMITTENTLY WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976 . . . . . . . . . . . . . . . . . . . 90

47 CHEMICAL ANALYSIS OF *S. polyrhiza* GROWN ON TEST CHANNELS PERIODICALLY FLOODED WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976 . . . . . . . . . . . . . . . . . . . 91

48 CHEMICAL ANALYSIS OF A MIXED STAND OF *S. oligorhiza* AND *S. polyrhiza* GROWN ON TEST CHANNELS PERIODICALLY FLOODED WITH DAIRY WASTE EFFLUENT, LATE, SUMMER 1976 . . . . . . 92

49 WATER QUALITY OF AERATED, STATIC LAGOON TEST CHANNELS SUPPORTING STANDS OF *S. polyrhiza*, LATE FALL, 1976 . . . . . . . 93

50 WATER QUALITY OF AERATED STATIC LAGOON TEST CHANNELS SUPPORTING A STAND OF *S. oligorhiza*, LATE FALL, 1976 . . . . . . 94

Number Page

51 WATER QUALITY OF AERATED STATIC LAGOON TEST CHANNELS SUPPORTING A MIXED STAND OF S. oligorhiza AND S. polyrhiza, LATE FALL, 1976 . . . . . . . . . . . . . . . . . . . . 95

52 WATER QUALITY OF AERATED STATIC LAGOON CONTROL CHANNELS, LATE FALL, 1976 . . . . . . . . . . . . . . . . . . . . . . . 96

53 BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE SAMPLES OF TEST LAGOONS 1 AND 2 . . . . . . . . . . . . . . . . . . . . . . . 97

54 BIOMASS OF S. oligorhiza, S. polyrhiza, AND A MIXED (M) CULTURE OF THE TWO HARVESTED FROM AERATED STATIC TEST LAGOONS, LATE FALL, 1976 . . . . . . . . . . . . . . . . . . . . 99

55 CHEMICAL ANALYSIS OF S. oligorhiza AND S. polyrhiza GROWN ON AERATED STATIC TEST CHANNELS RECEIVING DAIRY WASTE, LATE FALL, 1976 . . . . . . . . . . . . . . . . . . 100

56 WATER QUALITY OF AERATED, STATIC LAGOON TEST CHANNELS SUPPORTING STANDS OF S. oligorhiza, WINTER, 1976-77 . . . . . . 101

57 WATER QUALITY OF AERATED, STATIC LAGOON TEST CHANNELS, CONTROLS, WINTER, 1976-77 . . . . . . . . . . . . . . . . . . . . . 102

58 WATER QUALITY OF AERATED, STATIC TEST CHANNELS SUPPORTING STANDS OF L. gibba, WINTER, 1976-77 . . . . . . . . . 103

59 WATER QUALITY OF AERATED, STATIC TEST CHANNELS, CONTROLS, WINTER, 1976-77 . . . . . . . . . . . . . . . . . . . . . 104

60 BIOMASS OF S. oligorhiza AND L. gibba HARVESTED FROM AERATED, STATIC TEST LAGOONS, WINTER, 1976-77 . . . . . . . . . . 106

61 CHEMICAL ANALYSIS OF S. oligorhiza AND L. gibba GROWN ON AERATED STATIC TEST CHANNELS RECEIVING DAIRY WASTE, WINTER, 1976-77 . . . . . . . . . . . . . . . . . . . . . . 107

62 YIELD OF L. gibba, COVERED AND NON-COVERED AND A MIXED CULTURE (M) OF L. gibba AND S. oligorhiza FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, WINTER, 1977 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 109

63 PARTIAL CHEMICAL COMPOSITION OF L. gibba (G3) AND A MIXED CULTURE (M) OF L. gibba AND S. oligorhiza GROWN ON STATIC TEST CHANNEL RECEIVING DAIRY WASTE, WINTER, 1977 . . . . . . . . . . . . . . . . . . . . . . . . . . 110

Number Page

64 WATER QUALITY OF STATIC AND AERATED TEST CHANNELS SUPPORTING A MIXTURE OF S. oligorhiza AND L. gibba, WINTER, 1977 . . . . . . . . . . . . . . . . . . . . . . . . . 111

65 WATER QUALITY OF STATIC AND AERATED CONTROL CHANNELS, WINTER, 1977 . . . . . . . . . . . . . . . . . . . . . . . . . 112

66 BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM), OF COMPOSITE SAMPLES FROM MIXED SPECIES IN STATIC TEST CHANNELS, WINTER, 1977 . . . . . . . . . . 114

67 BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE SAMPLES FROM STATIC CONTROL CHANNELS, WINTER, 1977 . . . . . . . . . . . . . . . . . . 114

68 STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE MIXED SPECIES IN STATIC TEST CHANNELS, WINTER, 1977 . . . . . . . . . . . . . . 115

69 STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE SAMPLES FROM CONTROL CHANNELS, WINTER, 1977 . . . . . . . . . . . . . . . . . . 115

70 WATER QUALITY OF COVERED, AERATED AND STATIC TEST CHANNELS SUPPORTING L. gibba, WINTER, 1977 . . . . . . . . . . . . 116

71 WATER QUALITY OF NON-COVERED, AERATED AND STATIC TEST CHANNELS SUPPORTING L. gibba, WINTER, 1977 . . . . . . . . . 117

72 BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE SAMPLES FROM L. gibba STATIC, COVERED TEST CHANNELS, WINTER, 1977 . . . . . . . . . . . 118

73 BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE SAMPLES FROM L. gibba STATIC, NON-COVERED TEST CHANNELS, WINTER, 1977 . . . . . . . . . 118

74 STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE SAMPLES FROM L. gibba STATIC, COVERED CHANNELS, WINTER, 1977 . . . . . . . . . 119

Number Page

75 STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE SAMPLES FROM L. gibba STATIC, NON-COVERED TEST CHANNELS, WINTER, 1977 . . . . . 119

76 WATER QUALITY OF DUCKWEED COVERED TEST (AERATED) AND CONTROL (STATIC) CHANNELS, SUMMER, 1977 . . . . . 121

77 YIELD OF A MIXTURE OF L. gibba AND S. oligorhiza UNDER AERATION IN STATIC TEST CHANNELS FLOODED WITH DAIRY WASTE, SUMMER, 1977 . . . . . 123

78 CHEMICAL COMPOSITION OF A MIXTURE OF S. oligorhiza AND L. gibba UNDER LOW NUTRIENT AND AERATED CONDITIONS, SUMMER, 1977 . . . . . 124

79 ESTIMATED ANNUAL DRY WEIGHT YIELD PER HECTARE OF DUCKWEEDS PRODUCED ON LAGOONS RECEIVING DAIRY CATTLE WASTES, 1976-77 . . . . . 127

80 PARTIAL CHEMICAL COMPOSITION AND YIELD COMPARISONS OF SOME COMMON ANIMAL FEED PROTEIN SUPPLEMENTS AND DUCKWEEDS . . . . . 130

# LIST OF METRIC CONVERSIONS

## Length

1 centimeter = 0.3937 inch

1 inch = 2.540 centimeters

1 meter = 3.281 feet

1 foot = 0.305 meter

1 meter = 1.094 yard

1 yard = 0.914 meter

## Area

1 sq. meter = 10.76 sq. feet

1 sq. foot = 0.0929 sq. meter

1 hectare = 2.47 acres

1 acre = 0.405 hectare

## Weight

1 kilogram = 2.205 pounds

1 pound = .453 kilogram

1 metric ton = 2,204 pounds

1 short ton (2,000 pounds) = .907 metric ton

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# SECTION 1

# INTRODUCTION

The increased production of meat, poultry, and dairy products in the United States and the world has been paralleled by an increase in energy use and waste generation at a time of declining fossil fuel resources, increasing demand for water and air pollution control, and rapid world population expansion. Efforts to control population growth and develop additional energy sources are underway, but it is questionable at this time whether or not these programs will be achieved in time to thwart world-wide human starvation and serious deterioration of our environment. Many approaches are needed to obtain energy and increased food production to meet human demands. Of particular interest is the concept of energy production and nutrient reclamation through recycling of animal wastes to offset limited energy availability and to increase food resources. Development of a treatment system to economically generate energy and food from organic wastes should be applicable to livestock feedlot operations, and the food processing industry. With the increased confinement of animals into feedlots, the economics of extracting energy and nutrients from wastes becomes more attractive. The energy could be used in transportation of food and wastes, lighting, heating and cooling of air and water, refrigeration, etc. The nutrients could be easily made available for crop production, particularly if the crop is grown on lagoon systems adjacent to the feedlot.

In waste treatment emphasis has been placed upon breakdown of organic matter, but another equally significant aspect is that a very large percentage of the nutritive value of the feed remains in the manure. Recovery of these nutrients could be of considerable significance.

The use of aquatic plants to recover waste nutrients, though not a new concept, is in the early stages of development. Emphasis in lagoon-aquatic plant systems is not so much on treatment, as management. The management concept holds that a variety of products are available within the manure, or can be produced from proper manure management. Methane, of course, can be derived directly from the manure. Feeds can be derived both directly (refeeding of sludge) and indirectly (production of crops utilizing manures as fertilizers). Use of high-nutrient aquatic systems (lagoons) offers possibilities for developing a variety of products, but awaits the imagination of aquatic biologists for development.

This study reports on the use of duckweeds (family Lemnaceae) as part of a waste management system for a dairy farm. Emphasis was placed on evaluation of the plants in waste water renovation and as a feed ingredient for dairy cows. The rapid growth, high feed value, ease in harvest, cold tolerance, and ability to culture the plants adjacent to the feedlot offer several advantages over other aquatic plants as part of a waste management scheme.

The value of lagoon systems over land application in high rainfall areas are evaluated in this study, and a proposed scheme for an integrated waste management - energy efficient system is presented.

## SECTION 2

## CONCLUSIONS

### WATER QUALITY

Even under high manure loading rates duckweed covered channels showed greater reductions in Total Kjeldahl Nitrogen (TKN), ammonium ($NH_4^+$) and phosphorus (P) than controls. Under proper lagoon management where loading rates are controlled to minimize solids flow into lagoons duckweeds appear to offer treatment advantages.

Removal of phosphorus by duckweeds, though superior to control lagoons, was sufficiently low to consider another mechanism of removal. If duckweeds are to be used in the treatment, clonal selection for phosphorus concentration may be required.

With a multistage lagoon system and better control of loading rates than in our study, duckweeds should be capable of removing most nitrogen (N). Under the conditions of this study, duckweed-covered lagoons averaged about 20% greater nitrogen removal than controls. Ammonium was apparently the dominant form of N available to duckweeds.

### DUCKWEED PRODUCTION

The clones of *Spirodela oligorhiza* used in this study exhibited greater growth in the spring and fall, *S. polyrhiza* in the summer, and *Lemna gibba* (clone G3)* grew best during the fall, winter, and spring. Use of other clones within each species may not perform equally well. Mixed cultures of the species grew as well as the monoclonal cultures. The average and maximum annual yield projected to a per hectare basis was 17,577 kg and 22,023 kg (dry weight) respectively. Aeration and circulation of the lagoon water did not increase growth, and there was some evidence that growth may be reduced under aerobic conditions.

Nutrient content of the duckweeds increased when lagoon nutrients were increased, particularly when the TKN in lagoons exceeded ca 20-30 mg/l. Mean crude protein content (dry weight) was 36%, and a maximum of 42.6% was recorded. The plants compare favorably with ingredients of high protein

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*Clone identification number for *L. gibba*. Plant supplied by Dr. William Hillman, Brookhaven National Laboratory, Upton, L.I., N.Y. 11973. Referred to as *L. gibba* throughout this paper.

feeds and appear economical to use. Preliminary studies on cattle growth, palatability, and milk quality utilizing duckweed showed no adverse effects. Further study is needed.

On an annual per hectare basis, nutrient uptake by duckweed showed removal of N from the manure of 15.5 mature dairy cows, P from 34 cows, and potassium (K) from 8.8 cows. L. gibba showed the ability to concentrate K. TKN decline in duckweed lagoons averages ca 1 mg/l/day.

Duckweeds performed well under apparent anaerobic conditions, but the layer of water associated with the duckweed may have been aerobic. Year round production appears possible, particularly if the water temperature can be maintained above freezing. High loading rates reduced duckweed growth only when solids appeared on the lagoon surface. Surface algae blooms occasionally appeared to affect duckweed growth, but the problem was not serious. There was little evidence of pests affecting duckweed production. Mosquito larvae (unidentified) were frequently associated with the duckweed, but were more prevalent when harvest was not routine and complete cover was not achieved. Higher growth rates were suggested under continuous flow conditions as compared to static, and higher yields (current study) under daily harvest as compared to intervals exceeding three days.

## BACTERIOLOGICAL

Due to overloading of the system with waste, fecal coliform and fecal streptococci were relatively high. The major genera of bacteria were Proteus, Bacillus, Achromobacter, Flavobacterium, Escherichia, Enterobacter, Micrococcus, and Pseudomonas. Salmonella organisms were isolated from surface samples only twice, and each time were associated with a turtle infestation. During aeration studies in which the sludge was suspended in the water column Salmonella was not recovered. Pathogenic bacteria were not found in the effluent (surface overflow) other than mentioned above. Further study is needed as it is possible the bacteria attached to the duckweed. However, preliminary feeding tests for growth, palatability, and milk quality failed to show symptoms of pathogen infection. In all cases the duckweed was washed with chlorinated water, or dried before feeding.

## FEEDING TRIALS

Duckweed is a high quality feed material and acceptable to dairy cattle when fresh or dried. The cattle consumed up to 75% duckweed in the diet. Washing apparently improved the palatability response, as did mixing with other feeds. No adverse effect was noted on growth and milk quality. The acceptability of the wet duckweed by cows will greatly enhance energy conservation in processing the plant, which requires only washing. Further study is needed, however, as these tests involved only 1 to 4 cows.

# SECTION 3

## RECOMMENDATIONS

The primary goal of this study was to obtain information on the value of duckweeds as part of a waste management system. Particular emphasis was placed on: (1) the growth performance and nutrient uptake of these plants under the worst possible lagoon conditions, e.g. extreme overloading of the system; (2) chemical, physical, and microbiological characteristics of duckweed-covered lagoons in comparison with conventional lagoons; and (3) the usefulness of duckweeds as an animal feed. Based on these studies the following factors were evident: Some duckweeds adapted easily to waste lagoons, grew rapidly, concentrated nutrients, improved waste treatment, and appeared to be useful as a feed ingredient. These results support the following recommendations.

1. Research should continue to define the lagoon management requirements for maximum duckweed production and nutrient extracting.

2. Although few pathogenic bacteria were associated with the harvested duckweed and surface water, they were present in the deeper water and sludge. Therefore, bacteriological studies should continue, with emphasis on controlling the loading rate and washing of duckweed with chlorinated water before feeding.

3. Research should be undertaken to develop a duckweed harvesting and transportation system, as current studies indicate that daily harvest will result in higher yields than harvesting at intervals longer than three days.

4. Studies should be initiated to genetically engineer clones of duckweed with higher growth rates and nutrient value, cold tolerance, reduced water content and other desired characteristics for various uses.

5. The concept of agricultural waste management should emphasize nutrient recovery and utilization, energy production, development of marketable products and the production of acceptable quality water.

6. Lagooning of wastes for nutrient extraction and product development should take priority over land application in high rainfall areas, both for waste treatment and crop production. It is doubtful that yields of land-based protein crops will compete with aquatic plants such as duckweed.

7. Economic evaluations are needed for integrated waste management - food production schemes suggested in this study.

8. TKN decline during the study averaged ca 1 mg/l/day. Based on this study lagoons with 40-50 mg/l TKN should be reduced to less than 10 mg/l by the duckweed system within 30 to 40 days. However, shallow lagoons (< 0.3 m) and their management should be considered in a waste management scheme with duckweeds and other aquatic plants. Retention times could be greatly reduced, treatment improved, and plant yield increased.

9. Phosphorus reduction needs further study, as the uptake by duckweeds was not sufficient to meet strict water quality standards (of about 0.1 mg/l). Proper lagoon management may improve upon removal of this plant nutrient.

# SECTION 4

# TECHNICAL BACKGROUND

The needs to provide food for a hungry world and curb pollution are becoming increasingly difficult to achieve due to the human population growth, consumption, and limited available energy sources. Animal waste utilization offers good potential for generating energy and food.

TABLE 1 shows the estimated number of animal stocks grown under confinement in the United States which could be utilized to meet energy, food, and product demands. Development of our renewable waste resources through integrated recycling and use systems may well improve the economics of water and air pollution control.

Public reaction to water pollution and the general deterioration of the environment makes it mandatory that new technology be developed to resolve the ever growing mountains of pollutants caused by the increasing needs for food production. Waste resulting from heavily populated urban areas, mass production and the crowding of livestock and food processing plants near these areas has exceeded the capacity of present waste disposal systems. This aspect becomes even more graphic when one considers that livestock alone presently generates an estimated 2 billion tons* of wastes yearly (Anonymous, 1970).

Livestock has become highly concentrated in that some beef feedlots may contain up to 50,000 animals, dairy farms may have 5,000-8,000 animals on very limited acreage, poultry farms up to 1,000,000 birds and swine operations as many as 1,500. These wastes were formerly returned to the land, but the associated costs were more expensive than utilization of commercial fertilizers. In addition there is not sufficient available land near these concentrations of livestock to absorb the wastes produced without causing excessive pollution of waterways from runoff. The net result often is excessive nutrification and rapid eutrophication of surface and ground waters.

There is a current interest in land application of livestock waste rather than using lagoons for biological treatment of these animal wastes. Most of the research work on lagoons has been on the engineering aspects of

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*See LIST OF METRIC CONVERSIONS. In order to insure accuracy of the referenced authors, conversion of the reference data to metric system was not made.

TABLE 1. NUMBER OF ANIMALS MAINTAINED UNDER FEEDLOT CONDITIONS[a]

| Animal | Number of animals[b] |
|---|---|
| Dairy | $11 \times 10^6$ |
| Poultry (layers) | $478 \times 10^6$ |
| Poultry (broilers) | $1,000 \times 10^6$[c] |
| Swine | $61 \times 10^6$ |
| Turkey | $90 \times 10^6$ |
| Beef | $14 \times 10^6$ |
| Sheep | $4.2 \times 10^6$ |
| Ducks | $1.9 \times 10^6$ |
| Horses | $0.3 \times 10^6$ |

[a] Extracted from data compiled by Hamilton Standard (1973) for the EPA to serve as a guide for effluent management for the feedlot industry.

[b] These figures do not include animals in pasture.

[c] Data on poultry obtained from Louisiana State University Poultry Science Department.

the system, a carry-over from municipal lagoon design, but biological factors related to microbial activity have not been studied in detail. Unfortunately, the acceptance of lagoons and their use on farms has preceded definitive studies on their ability to adequately degrade animal waste and produce an acceptable effluent. This lack of knowledge of lagoon function, and how to control the system may have stimulated recent interest in land application. Lagoons are often extolled for the efficiency of biochemical oxygen demand ($BOD_5$) reduction (as high as 85%), but little mention is made of the fact that there frequently will be effluent flow, or the volume of effluent and its pollutional load. In high rainfall areas runoff and effluent flow is the rule, not the exception. The discharge contains organic and inorganic nutrients.

It is general knowledge that the primary means of handling livestock waste has been to return the waste to its original source -- the soil. Experiments (Russell, 1961; Whie and Holben, 1921; Wiancke, Walker, and Mulvey, 1935) have been conducted in which animal wastes were shown to be satisfactory sources of plant nutrients. The application of wastes on certain soils has proved to be an effective filter in removing pollution from water supplies. Overman, Hortenstine, and Wing (1970) have reported that the use of soil to reclaim dairy waste waters before discharge into receiving streams resulted in a stabilized effluent. McCaskey, Little, and Rollins (1971) reporting on the application of liquid and dry manure to soil indicated that a slightly higher BOD than the suggested maximum of 30 ppm for runoff was present when 2.4 tons of raw wet solids were applied per acre per month. When dry wastes were applied 1.5 to 2.3 times the recommended BOD was observed in the runoff from the application of 12.8 tons of wastes per acre per month. These studies were made on sandy type soils which are open soils that would provide filtering action. However, soils in the Mississippi River flood plain and Gulf coast areas, are mainly clay type soils and filtering rates are greatly reduced. High rainfall further complicates the tight soil problem.

In the incorporation of vascular aquatic plants into the biological treatment process, three distince advantages are evident: (1) An additional biological system is added to existing biological processes and should further augment waste treatment. (2) Since most chemicals in animal waste lagoons that impair water quality are in solution, large quantities leave the lagoon in the effluent. Those that are trapped in the bottom sediments must ultimately be removed by dredging, processing and transportation to another location. Utilization of aquatic plants could provide a method of removing these nutrients continuously and possibly reduce or eliminate the need for periodic dredging. (3) The lagoon owner receives no economic benefits from lagoon systems at present. Due to the high nutritional value of some aquatic plants and the ease of harvesting, the owner could receive economic benefits by utilizing these plants as animal feeds and reduce the labor and cost of spreading waste on land. It is imperative that the long-range research goals be designed to continue to refine and improve on waste treatment processes to insure continued multiple use of water resources and to provide economic benefits from the treatment process.

In Louisiana, over 200 lagoons are now in operation. Nine other southern states probably have similar numbers. The design recommendations allow for overflow. Redesign of these lagoons to prevent overflow will be expensive. A treatment process utilizing aquatic plants may improve the water quality sufficiently to allow the existing lagoons to remain unchanged. The overflow water may be of acceptable quality to recycle as wash water, or if used in irrigation (at certain times of the year) be of such quality that no contamination of surface waters will occur. However, these ten states have high annual rainfall with much of it coming in a few months. In Louisiana it is not uncommon to have from 25 to 40 cm in one month. The use of lagoon water for irrigation is of little value as a waste treatment technique during rainy seasons as is land application of manure. The alternatives are to redesign each lagoon to have no overflow, or use a combination of irrigation (during dry periods) and recycling of the effluent as wash water during the rainy season. The latter approach may be economically more desirable, particularly if the aquatic plants can be economically utilized.

The use of lagoons has been of three types, based on biological activity, namely, anaerobic, aerobic, and a combination of the two. Most of the research on these systems to date have been conducted on swine and poultry wastes.

Anaerobic plus aerobic lagoons have been under considerable study by several investigators (Anthony, 1969; Foree and O'Dell, 1969; Shmid and Lipper, 1969; Sobel, 1969; Vickers and Genetelle, 1969) and appear to offer excellent possibilities for livestock waste disposal. Loading rates, separation methods, microbiological characteristics, climate and design details are under preliminary study, but must be examined in detail to provide efficient economical operational procedures.

It is conceivable that a combination system of waste screening, anaerobic and aerobic breakdown may offer possibilities for the requirements needed for a non-polluting stabilized effluent. Continued research will be necessary to define the specific problems associated with collection, treatment and nutrient losses. The ultimate goal is to design systems that will stabilize the undesirable materials and allow re-use of valuable nutrients at a low cost to the farmer.

It has been emphasized that research on more effective techniques to remove and/or recovery of mineral nutrients from effluents prior to release in natural waters is needed (American Chemical Society, 1969). Animal and municipal waste lagoons convert large quantities of organic nutrients to inorganic nutrients and minerals. Great quantities of inorganic nutrients leave the lagoon system with the effluent and no practical or economically beneficial method of extraction has been developed. Inorganics that are trapped in the sludge must ultimately be disposed of as it is necessary to periodically dredge out the sludge. Processing or disposal of sludge is expensive. Incorporation of aquatic plants into lagoon systems may reduce the sludge load and remove large quantities of nutrients dissolved in the water column.

Although considerable research has been conducted on the use of aquatic plants in waste treatment, biomass production, and as an animal feed, little success has been realized in developing an economically feasible system for any of these uses. However, use of aquatic plants in the family Lemnaceae (duckweeds) has been largely ignored as a food source until recently (Bhanthumnarin and McGarry, 1971; Culley and Epps, 1973; Truax et al., 1972; Sutton and Ornes, 1975; Harvey and Fox, 1973). These studies suggest that duckweeds hold high potential as an animal feed. Growth rates in the laboratory and under field conditions exceed traditional agricultural crops in biomass production, and have a high nutrient quality (up to 45% protein). Water content of certain duckweeds varies from 90-94%, harvest techniques are available, and some plants tolerate light freezes and actively grow during mild winters. The several criteria listed below for selecting specific aquatic plants for waste treatment are best fulfilled by duckweeds. Aquatic plants should (1) be easily harvested, (2) be low in water content, (3) possess a high protein content, (4) have a low fiber and lignin content, (5) have a high mineral absorption capability, (6) have an extended growing and harvesting period, (7) be non-toxic to human and domestic stocks, (8) be easily processed, and (9) few pests. Based on the studies by Truax et al. (1972) and Culley and Epps (1973), duckweeds fulfill criteria 1, 3, 4, 5, 6, and 9 and partially fulfill criteria 7 and 8. Based on the criteria it is evident that not only can duckweeds be used for extended periods to take up nutrients, but they can also be harvested and the energy and nutrients in the plants recycled back into animal feeds. The possibility of economic benefits resulting from utilizing duckweeds makes them extremely attractive in water treatment processes.

Joy (1969) and Hillman (1957; 1961) state that a variety of organic compounds have been used as duckweed culture media, indicating their ability to utilize organic compounds for growth, even in the absence of photosynthetic light levels. Joy obtained greatest growth rates in medias consisting of minerals, sucrose, and hydrolyzed casein. Thus, duckweed may function not only to tie up inorganic fractions in a lagoon system, but may aid bacteria by removing organics. Therefore, it may be possible to load a lagoon system with organic wastes at a higher rate than could be afforded with present biological systems in use.

Utilization of duckweeds should be applicable to a variety of waste lagoons, particularly those involving treatment of (1) all types of animal excrement, (2) food processing plants such as canning, meat and poultry, sugar mills, rendering and leather plants, paper mills, etc., and (3) industries involved with release of metal wastes, radioactive materials, fertilizers, and possibly insecticides.

## LAGOONING VS. LAND APPLICATION

The agricultural industry faces a need for an environmentally acceptable method of livestock waste disposal. Due to high labor and land cost, livestock operations are moving to more confined systems. The concentrating of larger groups of animals into smaller areas causes increased problems in waste management.

In 1969 there were 604 dairies in Louisiana with 20-30 cows. These farms accounted for approximately 35% of all dairy farms in the state. There were 835 producers with herds of 50-100 cows, representing 49% of the total statewide. From 1964 to 1969, nationally, the farms in the range of 50-100 head increased from 37,601 farms (16% of the total cows) to 38,457 farms (22.6% of total cows). In the range of 100-more head per farm, in 1964 there were 8,846 farms (0.8% of total cows), nationally. In Louisiana farms of 100-more head, the increase was from 235 farms (18.6% of total cows) in 1964 to 275 farms (33.0% of total cows) in 1969. In 1965 the average herd in Louisiana was 68 cows; by 1975 this figure had increased to 92 cows. The forecast for the future is a movement to herds of 300-500 cows being housed in concentrated feedlot situations with little cropland or pasture available for waste disposal.

Because of growing concern over pollution, the development of an acceptable waste disposal system is of high priority. The waste produced by one cow equals that of 16.4 humans, similarly 100,000 layers produce waste equivalent to that of 1,500 people (Eby, 1962). Each person is supported by 2 laying hens, 4 broilers, one-half turkey, one-half cow, one-third pig, one-tenth sheep, and several other smaller animals too numerous to mention, producing in excess of 6 million tons of manure daily (Turner, 1970).

## Scrape and Spread Method

The conventional system of handling waste requires that the confinement area be scraped daily and manure loaded directly into the manure spreader. When the spreader is fully loaded, manure is spread in fields adjacent to the dairy. Daily manure production of a 1400 pound dairy cow was reported by a North Carolina study, Drigger et al. (1973), as 118 pounds per day. For a 150 cow herd two loads of a manure spreader is required daily to remove the waste (ca 4.5 metric tons per load). This requires two persons about three hours each day in good weather and field conditions. During inclimate weather the procedure quickly becomes restrictive due to the inability to move the manure, stockpiling, etc. Efforts to spread the manure frequently result in damage to pasture drainage contours. Not only is a health hazard produced by stockpiling, but when weather conditions improve, personnel are tied up an excessive amount of time to remove the stockpile. In short, the scrape and spread method is not very efficient.

The amount of manure/hectare that can be applied varies with the type of soil, the crop, and the area of the United States. Soils with a high percentage of clay, such as those in Louisiana, do not lend themselves to high levels of manure application due to low percolation capacity resulting in soil saturation and runoff. The amount of N that can be utilized by a crop also places a limit on the quantity of waste than can be applied to a given soil. Some average values include: feedlot manure on grain sorghum is 25 metric tons/hectare every 3 years while that for irrigated corn silage is 250-320 metric tons/hectare. Since irrigation water decreases the ammonium and salt concentrations in soils, larger amounts of manure may be added under these conditions, but irrigated crops are impractical in certain high rainfall areas of the nation (Loehr, 1977).

## Lagooning

A basic lagoon waste handling system consists of an aerobic pond and an anaerobic pond. These ponds should be designed to meet specifications recommended by the Soil Conservation Service (SCS) if treatment only is desired. The lagoon as a natural waste oxidizing system has been used for many years, and today its use as a primary method of waste disposal is widespread. This use of lagooning is attributable to low cost of construction, ease of operation, and low maintenance. Manure is usually scraped once each day from the holding area into the anaerobic lagoon. Anaerobic lagoons may be loaded in excess of 448 kg BOD/ha/day. The aerobic or facultative pond is usually designed for loading of 56 kg/BOD/ha/day with effluents from the aerobic pond having a BOD concentration of 20-40 mg/l. Lagoons normally achieve 50-90% BOD removal, depending on loading, retention time, and whether or not solids are removed prior to discharge (Loehr, 1977). Incorporation of aquatic plants into the system should improve treatment. However, in terms of waste management, optimum recommended lagoon designs are unknown. The use of SCS recommendations on existing farms should probably be utilized until management techniques are defined.

## Cost Review

A study was undertaken at Michigan State University by Hogland et al. (1972) to determine manure handling practices in use and to develop investment and cost data. Investments in complete manure handling systems ranged from $80 per cow for gutter-spreader system to $190 per cow for a liquid manure-handling system.

Buxton and Zeigler (1974) estimated added investment and annual cost dairy operators would incur in controlling runoff, wash water, and added water from major storms. It was concluded that the cost of pollution control would be borne by the dairy farmer in the short run. Small farms might be forced out of production or forced to expand and adopt more efficient housing and milking technology.

In a study of Louisiana dairymen conducted by Hromadka (1976), it was found with an average herd size of 110 cows the investment per cow was $21.38 for a lagoon system (ramp, anaerobic lagoon, and aerobic lagoon) vs. $47.98 per cow for a scraper-loader-spreader system.

Alternative waste handling systems were examined in a study at the University of Tennessee conducted by Henderson and Bauer (1973). Labor requirements for liquid, conventional, and irrigation systems were similar. The lagoon system required no labor beyond scraping the loafing area.

Miner (1975) states that, "Currently, most animal waste is applied to agricultural land; liquids are irrigated, and solids or slurries applied by a spreader. The term, disposal area, has gained undesirable currency. If long-term pollution of surface or ground water is to be avoided, rates of manure application must match the assimilation capacity of the site; that is, capacity that minimizes release of pollutants. In some arid areas of the country, this matching may allow very high manure application for one year, followed by several resting years for assimilation. Humid areas with regular rainfall will not be able to follow such a practice because of nutrient infiltration or runoff. The concept of disposal areas is not compatible with the maintenance of soil productivity."

The use of manure for land application has specific disadvantages in certain areas of the United States, such as Louisiana. Due to the high annual rainfall (approximately 150 cm/year) it would be impossible at certain periods of the year to spread manure and the quantity would be limited, also there would be a high percentage of runoff. The need for holding areas brings with it the need for least cost operation. A lagoon system offers the best of alternatives, with a large holding capacity for manure and runoff, low investment, ease of operation, and ability to irrigate or fertilize with the effluent, if the producer has the need or desire to do so. Catching of surface runoff water from the confinement area can not be over emphasized due to the large volumes of water involved from rainfall. A lagoon system seems to be the easiest method for the average producer to comply with regulations. In confinement operations the use of water from the aerobic lagoon for washing down the loafing area offers a "closed system" alternative, bringing the producer in full compliance with existing regulations of "no point discharge."

## SECTION 5

## OBJECTIVES

The long-term goals of this research at Louisiana State University are to determine if efficient methods of livestock waste treatment and reduction in the use of energy can be achieved by recycling waste nutrients from lagoon systems back into animal feeds. Dairy cattle wastes are being used as the model for treatment of livestock waste.

This particular study was designed to incorporate duckweeds into the lagoon treatment process to determine if (1) waste treatment could be enhanced, and (2) a sufficient quantity of duckweeds with acceptable nutritional feed value could be produced on the system. The following objectives were considered.

1. Determine the chemical, physical and microbiological composition of lagoon waters receiving dairy cattle wastes.
2. Determine the effluent water quality of lagoons with and without duckweed cover throughout the year.
3. Determine the chemical composition, rate of production, and harvest rate of duckweeds, and relate these values to changes in water quality.
4. Obtain information to establish optimum loading rates for maximum growth and nutrient removal by aquatic plants.

# SECTION 6

# METHODS

## CHARACTERISTICS OF STUDY AREA AND HERD

This study was conducted at the Dairy Product Teaching and Research Center, a research unit of the Department of Dairy Science and the Louisiana Agricultural Experiment Station in Baton Rouge, Louisiana. A 370 acre (150 ha) farm, located off campus is used to house and care for approximately 350 animals (115 milking cows). The lactating herd is maintained in a dry lot adjacent to a four lagoon complex. The lagoons (Figure 1) receive liquid wastes from the milking parlor, wash-down from the holding areas, and solid waste and rainfall on the loafing area. Solid waste was added as needed.

Solid wastes were pushed into the lagoon via the loading ramp. Liquid wastes flowed by gravity to the collection sump, where fibrous materials were screened from the liquid. A two horsepower sewage pump conveyed the liquid through a 4-inch (10 cm) PVC plastic pipe to the stage 1 anaerobic lagoon where some solids settle-out and biological activity initiates break-down of the waste. Retention time was only a few days. Effluent passed by gravity through a 2-inch (5 cm) PVC pipe to test lagoons 1 and 2, or the collection pit. Each test lagoon was subdivided into 8 test channels. Effluent flow could be individually directed into and diverted from each of these channels.

Each test channel was ca 2.7 m wide and when fully flooded up to 10 m in length. Surface area for each channel when full was about 26 $m^2$. Maximum water depth was about 1.3 m. Movable vertical drain lines at the opposite end from the valves could be adjusted to control the water depth.

Partitions dividing each test channel (8/lagoon) were made of wooden fencing material and lined with nylon reinforced plastic and embedded in the lagoon floor. Walkways were constructed over each partition to allow sampling at various locations within each lagoon.

In order to control harvesting of duckweed within each test channel a plastic partition was placed midway across each channel. This partition rested on the floor of the lagoon and was simply raised to the surface so only one-half of the duckweed could be removed, leaving the other half for regrowth.

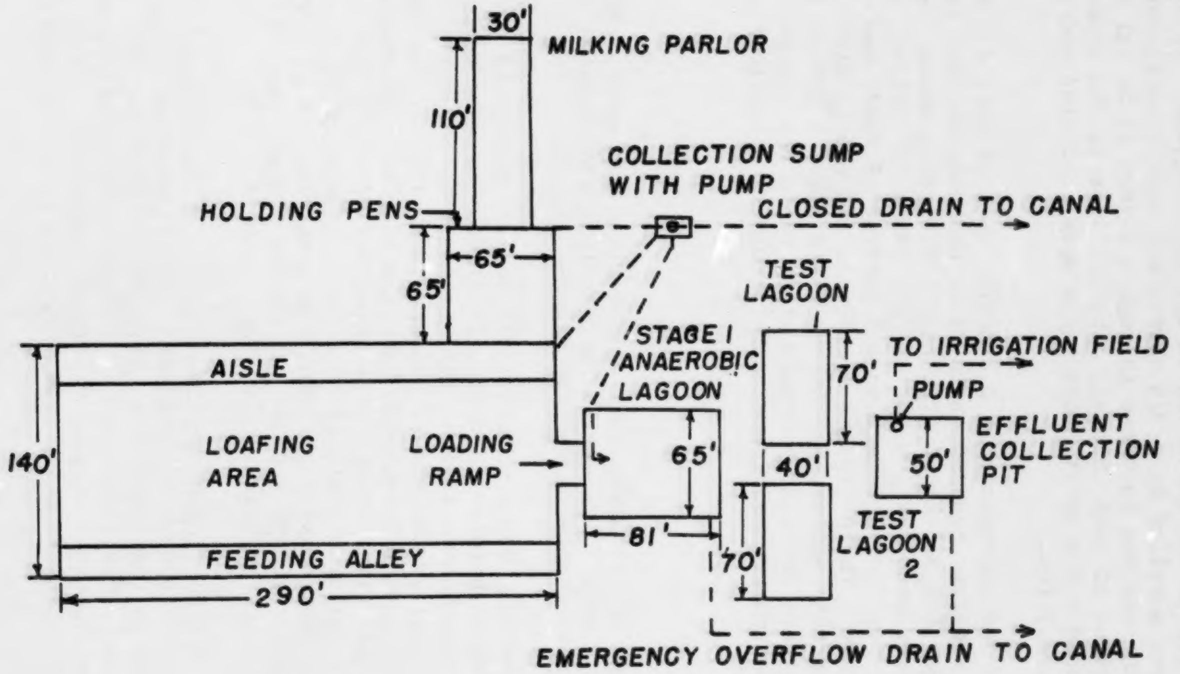
30'
MILKING PARLOR
110'
COLLECTION SUMP
WITH PUMP
CLOSED DRAIN TO CANAL
HOLDING PENS
65'
65'
TEST
LAGOON
STAGE I
ANAEROBIC
LAGOON
TO IRRIGATION FIELD
70'
PUMP
AISLE
EFFLUENT
COLLECTION
PIT
140'
LOAFING
AREA
LOADING
RAMP
65'
40'
50'
81'
TEST
LAGOON
2
FEEDING ALLEY
70'
290'
EMERGENCY OVERFLOW DRAIN TO CANAL

Figure 1. Campus dairy-herd facility showing location of feedlot and milking parlor. Scale 1" = 100'.

Thermocouples were placed in each test channel to monitor temperatures at various depths.

An aeration system was installed in the test lagoons. Pressurized air for this system was supplied by a 3/4 horsepower electrically-operated compressor, and the air was conveyed through a 1-inch (2.54 cm) PVC plastic pipe along the bottom of each channel. The air line in the channel had ca 1 mm holes spaced 0.5 m apart. All tests were conducted under aerated and non-aerated conditions.

Each cow was fed approximately 2.3 metric tons of hay, 6.4 metric tons of silage, and 2.7 metric tons of grain (feed concentrate) per year to produce an average of 5,578 kg of milk/year. Estimated energy requirements to maintain the herd were $794.2 \times 10^9$ cal/yr, $561 \times 10^9$ cal/yr, and $21.9 \times 10^9$ cal/yr for feed; production and harvest of feed; and waste removal respectively. These figures are low as all energy expenditures could not be accurately estimated. An estimated $419.4 \times 10^9$ cal/yr are produced in the milk. The energy input-output ratio for the dairy herd is about 4 to 1 if the energy requirements for building operations are included.

## TECHNIQUES

All three lagoons were initially filled with city water. Samples were taken after several days to determine the physical and chemical characteristics of water exposed only to soil within the lagoon.

Chemical, physical, and microbial analyses was monitored using standard analytical techniques (Horwitz, 1975) and Standard Methods (American Public Health Association, 1971). Analytical quality control was periodically monitored by the Animal Production Section of the USEPA Robert S. Kerr Environmental Research Laboratory at Ada, Oklahoma. Water analyses included pH, temperature, total Kjeldahl Nitrogen (TKN), phosphorus (P), potassium (K), calcium (Ca), nitrate ($NO_3$), ammonium ($NH_4$), total suspended matter (TSM), volatile, fixed, and total residue (VR, FR, and TR), dissolved oxygen (DO), biochemical oxygen demand ($BOD_5$), chemical oxygen demand (COD), and total organic carbon (TOC).

The lagoons were monitored bacteriologically. Test channel samples were taken from the outlet which received surface water overflow. Microbiological analyses included standart plate counts (SPC) at 20°C and 32°C, anaerobic counts (AC), fecal coliform (FC) and streptococci counts (FC). Deatiled methods as outlined by Bergey, Breed, and Murray (1957) and the Society of American Bacteriologists (1957) were utilized in characterization of micro-organisms.

Chemical analysis of the plants included such determinations as total N, P, K, Ca, fats, fiber, ash, moisture, nitrogen-free extract (NFE), and TOC following the techniques of Horwitz (1975), with periodic analytical quality control monitoring by the Animal Production Section located at Ada, Oklahoma.

Throughout the study, several tests were conducted under maximum loading rates of manure. The stage 1 anaerobic lagoon was deliberately overloaded to create as high a nutrient load as possible in order to determine duckweed performance under the most adverse conditions. Under these conditions, several species of Lemnaceae were initially introduced to determine their ability to adapt to the system. The criteria used in selecting the species for additional study were: (1) recovery response of the plants after collecting, transporting, and stocking on water with chemical characteristics quite different from collection sites; (2) rate of recovery; (3) evidence of rapid growth; (4) chemical characteristics; and (5) evidence of cold tolerance, based on growth during winter months. The duckweeds evaluated were species of _Lemna_, _Spirodela_, and _Wolffia_. Two other plants with similar growth forms, _Azolla_ _caroliniana_ (Salviniaceae family) and a liverwort, _Ricciocarpus_ _natans_ (Ricciaceae family) were evaluated. The former was selected because species within the genus are known to have a symbiotic relationship with a blue-green algae that fixes nitrogen (Ashton and Walmsley, 1976), and could possibly enhance nitrogen recovery or protein content of the _Azolla_.

Cultures of the various duckweeds were maintained at the experimental fisheries research station in earthen ponds. A sufficient quantity of a species was stocked on each test channel to allow rapid covering. When full coverage was achieved, one-half of the plants were removed, allowed to drain for 24 hours in perforated plastic buckets and then weighed. Samples were then washed, oven dried for 18 hours at 95°C and then chemically analyzed. Monthly harvest rates were estimates based on the quantity of plants (wet and dry weight) removed from each test channel.

Tests were conducted under static conditions, i.e. each test channel was filled to capacity with waste water and then the valves cut off. Each channel was flooded from two to four days in order to insure similar chemical characteristics existed in each channel before testing. Water quality samples were taken and then the duckweed stocked. Control channels without duckweed were used to compare performance. Records were maintained on changes in surface area due to evaporation and figured into the calculations of total harvest per channel. Volume changes in the channels were also calculated and related to changing water quality. Chemical, physical, and microbial analyses were conducted at each harvest or more frequently. Tests were conducted during each season through the year. During winter some channels were covered with clear plastic and duckweed growth was compared with that in non-covered channels.

Preliminary feeding trials were conducted with dairy cattle depending on the quantity of material available. Emphasis was placed on the palatability and digestibility of duckweed (wet and dry).

# SECTION 7

# RESULTS

## PLANT SCREENING

Studies involving the use of duckweeds were initiated July 1975. Initial studies involved screening species of duckweed and other plants with similar growth forms. Clones of the following duckweeds were evaluated: *Spriodela oligorhiza*, *S. polyrhiza*, *Lemna perpusilla*, *L. gibba*, and *Wolffia* spp. In addition, *Azolla caroliniana* and *Ricciocarpus natans* were evaluated.

The latter two species and *L. gibba* showed evidence under field conditions of cold tolerance, but growth of the water fern and liverwort was sporadic over a two month period. *Spirodela oligorhiza* and *S. polyrhiza* were selected for further study due to high animal feed quality characteristics, apparent rapid growth, some evidence of cold tolerance, and their ability to adapt quickly after considerable abuse in collecting and transporting. Sustained growth appeared to occur through the summer and fall months. *L. gibba* was selected also, primarily due to its active growth during winter months. Little growth was evident during the summer. *L. perpusilla* and *Wolffia* adapted poorly to the system and the former gradually disappeared. *Wolffia* maintained itself in low numbers and showed evidence of sporadic rapid growth, particularly in cool weather. Both *L. perpusilla* and *Wolffia* were dropped due to their slow adaptation to the system.

TABLE 2 shows some chemical vlues obtained from the *Ricciocarpus* and *Azolla* grown under lagoon conditions, indicating possible value as an animal feed ingredient. Due to sporadic growth further study was terminated. Values for the species used in followup studies are shown in other tables.

## WASTE TREATMENT STUDIES

### Characteristics of Various Components Used in the Study

Chemical values were obtained for the lagoon water and soils, animal waste and duckweeds prior to the introduction of waste. Periodic sampling of the animal waste was continued throughout the study. TABLE 3 shows the chemical content of two duckweeds maintained in low nutrient systems before culture. TABLE 4 shows water, soil, and manure when the lagoons were completed and ready for loading.

TABLE 2. CHEMICAL COMPOSITION OF PARTIALLY DRIED *Ricciocarpus natans* AND *Azolla caroliniana* GROWN ON DAIRY WASTE LAGOONS UNDER WINTER CONDITIONS, JANUARY, 1976

|  | % |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
|  | Crude Protein[a] | Fat | Fiber | Moisture | Ash | N | P | K | Ca | Lignin |
| *Ricciocarpus* | 30.0 | 4.0 | 8.2 | 6.4 | 9.5 | 4.8 | 0.69 | 2.0 | 0.83 | 11.9 |
| *Azolla* | 30.2 | 3.8 | 9.8 | 5.9 | 18.6 | 4.8 | 0.74 | 5.6 | 1.1 | 5.1 |

[a] Protein equals N x 6.25.

TABLE 3. CHEMICAL CHARACTERISTICS OF DUCKWEED MAINTAINED IN LOW NUTRIENT PONDS BEFORE USE IN LAGOONS RECEIVING DAIRY CATTLE WASTE

| | % of dry matter | | | | | | |
|---|---|---|---|---|---|---|---|
| | Crude[a] Protein | Fat | Fiber | Ash | N | P | K |
| *Spirodela oligorhiza* | 13.5 | 2.3 | 12.5 | 16.3 | 2.11 | 0.56 | 2.0 |
| *Spirodela polyrhiza* | 12.9 | 2.5 | 18.2 | 12.7 | 2.07 | 0.54 | 2.4 |

[a] Nitrogen x 6.25.

TABLE 4. CHEMICAL ANALYSIS[a] OF CITY WATER, LAGOON SOILS, AND MANURE

| | Water (mg/l) | | Soil (mg/kg) dry | | Manure (mg/kg) dry |
|---|---|---|---|---|---|
| | 6/14/76 | 9/1/76 | 6/14/76 | 9/1/76 | 6/14/76 |
| COD | < 5 | < 5 | --- | --- | $1.2 \times 10^6$ |
| TKN | .70 | .70 | --- | --- | $3 \times 10^4$ |
| Phosphorus | .28 | .24 | --- | --- | 8200 |
| Calcium | .50 | 1.20 | 5600 | 8400 | $22 \times 10^3$ |
| Magnesium | 4.50 | .06 | 2400 | 2500 | 6200 |
| Sodium | 88.0 | 76.0 | 330 | 410 | 950 |
| Potassium | .40 | .30 | 2800 | 3200 | 4600 |
| Chloride | 9.80 | 7.40 | --- | --- | 3500 |
| Cobalt | < .01 | < .01 | 4.90 | 7.30 | .80 |
| Copper | .02 | .02 | 5.10 | 3.50 | 11.0 |
| Iron | .10 | .00 | 11000 | 8700 | 1900 |
| Manganese | < .05 | .00 | 260 | 240 | 110 |
| Zinc | .03 | < .02 | 64 | 66 | 82 |
| Aluminum | --- | --- | 9600 | 9300 | 3400 |
| Nitrate | .03 | .12 | --- | --- | < 7.50 |
| Nitrite | < .03 | < .03 | --- | --- | 9.00 |

[a] Average of several composite samples taken throughout the study.

From February 26 to April 6 two samples from the stage 1 anaerobic lagoon were analyzed, one a composite sample taken at three points in the lagoon and the second at the outlet. Analyses of these two samples indicated there was little difference between the, thus the outlet was sampled as an accurate measure of the water quality of the stage 1 anaerobic lagoon and the effluent entering the test channels.

TABLE 5 presents $BOD_5$, COD, total, volatile, and fixed residue of the stage 1 anaerobic lagoon outlet. The general trend was an increase in all values as the lagoon became loaded with organic matter. The range of $BOD_5$ values was 415 mg/l on March 3 to 814 mg/l on April 4 and the COD range was 1180 mg/l on February 25 to 2701 mg/l on March 24. The total residue, volatile residue, and fixed residue showed the same general trend of increase until loading of manure was stopped. The total residue ranged from 937 mg/l on March 3 to 2730 mg/l on April 14, volatile residue from 595 mg/l on February 26 to 1504 mg/l on March 17, and the fixed residue from 220 mg/l on March 3 to 1278 mg/l on March 10. Variation may be attributed to the building and breakdown of the anaerobic lagoon crust.

TABLE 6 gives TKN and pH values in the stage 1 lagoon. The TKN suddenly increased with loading of manure, but the pH changed little. Over time, the TKN gradually increased while the pH declined but stabilized between 6.5 and 7.0.

The Standard Plate Count, Anaerobic Count, Fecal Coliform, and Fecal Streptococci counts are shown in TABLE 7. Little variation occurred in these counts during the entire testing period. The average values were: SPC, $2.8 \times 10^6$/ml; AC, $2.1 \times 10^6$/ml; FC, $2.6 \times 10^6$/100 ml; and FS, $2.0 \times 10^6$/100 ml.

## Test 1, Spring Treatment, 1976

To initiate the study, the test lagoons were filled with tap water in early January. Duckweeds were stocked February 24 and then waste water was introduced beginning March 10 and continued for 3 days until TKN values approximated values in the stage 1 anaerobic lagoon (Figure 1). Duckweed harvest and water quality analysis was initiated after the plants fully covered the test channels.

Preliminary tests showed that growth slowed once full coverage was achieved. Thus, duckweeds in channels that were slow in achieving full cover, in a few days would catch up with those that covered quickly. When similar plant biomass was observed one-half of the duckweed from each test channel was harvested by seining (April 8). Routine water quality, microbial, and duckweed analyses began on the day of harvest. The control channels did not contain duckweed. As we did not know how the system would perform, complete chemical analyses were not run. Each channel was sampled to obtain an idea of variability using TKN and pH as the standards. Samples were taken about 12 cm below the surface. Other values were from pooled samples. The data reflects water quality changes in channels tested only with _S_. _oligorhiza_. _S_. _polyrhiza_ did not achieve full coverage, and was therefore excluded.

TABLE 5. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE OF STAGE 1 ANAEROBIC DAIRY LAGOON EFFLUENT AFTER LOADING BEGAN (FEBRUARY 19, 1976)

| Date | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue |
|---|---|---|---|---|---|
| | mg/l | | | | |
| 2/25/76 | --- | 1180 | 1195 | 723 | 472 |
| 2/26 | --- | 1162 | 944 | 595 | 349 |
| 3/3 | 415 | 2105 | 937 | 717 | 220 |
| 3/10[a] | 540 | 2047 | 2714 | 1436 | 1278 |
| 3/17 | 483 | 2546 | 2728 | 1504 | 1224 |
| 3/24 | 585 | 2701 | 2624 | 1492 | 1132 |
| 3/31 | 678 | 2016 | 1912 | 1242 | 670 |
| 4/6 | 814 | 2320 | 2084 | 1422 | 662 |
| 4/14 | 667 | 2202 | 2740 | 1422 | 1318 |
| 4/22 | 533 | 1936 | 2416 | 1492 | 924 |
| 4/29 | 467 | 1665 | 2098 | 1440 | 658 |
| 5/6 | 557 | 1790 | 2013 | 1063 | 950 |

[a] Flow to test channels initiated and continued for three days.

TABLE 6. STAGE 1 ANAEROBIC LAGOON TKN AND pH VALUES

| Date | TKN (mg/l) | pH |
|---|---|---|
| 2/3 | 4.2 | 7.60 |
| 2/11 | 4.1 | 7.75 |
| 2/18 (manure loading initiated)[a] | 55.7 | 7.55 |
| 2/24 | 80.5 | 7.35 |
| 3/1 | 122.0 | 7.45 |
| 3/6 | 179.0 | 7.45 |
| 3/16 | 209.9 | 7.15 |
| 3/22 | 196.3 | 6.90 |
| 3/30 (manure loading ceased) | 123.5 | 7.00 |
| 4/4 | 129.1 | 6.55 |
| 4/15 | 123.5 | 6.55 |
| 4/27 | 119.4 | 6.80 |
| 5/5 | 105.9 | 6.80 |

[a] Approximately 3,651 kg added/day. Washwater from the loafing area and milking parlor were added daily throughout most of the year.

TABLE 7. STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE STAGE 1 ANAEROBIC LAGOON EFFLUENT

| Date | Standard Plate Count per ml | Anaerobic Count per ml | Fecal Coliform per 100 ml | Fecal Streptococci per 100 ml |
|---|---|---|---|---|
| | x $10^6$ | | | |
| 2/26/76 | 3.9 | 2.5 | 4.9 | 3.3 |
| 3/3 | 2.1 | 4.3 | 3.3 | 1.8 |
| 3/10 | 2.3 | 1.6 | 3.5 | 3.3 |
| 3/17 | 2.3 | 1.5 | 3.1 | 1.7 |
| 3/24 | 3.4 | 1.5 | 3.3 | 2.3 |
| 3/31 | 3.5 | 1.5 | 2.3 | 1.7 |
| 4.6 | 2.3 | 2.5 | 1.1 | 1.8 |
| 4/14 | 2.9 | 1.8 | --- | --- |
| 4/22 | 2.0 | 1.8 | 1.8 | 1.2 |
| 4/29 | 3.0 | 1.9 | 1.3 | 1.4 |
| 5/6 | 2.6 | 1.8 | 1.2 | 1.4 |
| Average Value | 2.8 | 2.1 | 2.6 | 2.0 |

During the test, we received 1.1 cm of rain (TABLE 8), thus water levels declined during the study. Water quality values shown in other tables have not been corrected for evaporation. Normal rainfall for April and May is 13.9 and 10.9 cm respectively. Had we received this much rain the water would have remained at about the original depth and water quality values would have been about 20% lower than shown. TABLE 9 shows the change in BOD5, COD, total, fixed and volatile residue for duckweed covered and control channels. The data show that the control and duckweed-covered channels varied in their response to the values measured. However, since only one control channel was available for comparison, care should be excercised in concluding a significant difference existed for any parameter. The data indicate that the duckweed-covered channels showed approximately the performance of control channels. Figure 2 depicts the changes graphically.

TABLE 10 shows bacterial counts for the duckweed and control lagoons. Data was not available when the test was initiated so the percent change could not be determined. Based on the final counts it appears that the duckweed-covered lagoons had lower bacterial levels than the control channels. These values were a little surprising due to the apparent lower phytoplankton levels in the duckweed channels. Reduced competition for nutrients by the phytoplankton in the test channels could result in higher bacterial levels, but the data shows higher bacterial counts occurred in control channels with higher phytoplankton levels.

The TKN reduction was similar in the test and control channels (Figure 3). Reduction in the control channel was 30%, and 29.5% for the test channel. The TKN declined at a rate of 1.26 mg/l/day under both conditions. However evaporation would serve to concentrate the TKN so the quantity removed daily was actually higher. Correction for water loss (ca 20% of the water volume) shows that about 41 mg/l of TKN was removed. Under normal rainfall at this time of year, this reduction would be expected.

At nutrient levels where the TKN values exceeded 110 mg/l it would require about 90-100 days retention time for nitrogen to be reduced to acceptable levels for release under static lagoons with no circulation, assuming the rate of decline was linear. However, chemical stratification was evident in the test channels and the lower values near the surface could have reduced the rate of duckweed growth.

Based on these data, loading rates for lagoons should be lower than used here. The question of immediate importance, however, is what happens to the nitrogen in both systems? All channels were anaerobic, indicating that much of the nitrogen was in the ammonium form or tied up in the biota. Since water samples were not filtered we were unable to determine the quantity in solution, and therefore available to the duckweeds. It is also conceivable that the top 2 or 3 cm of the duckweed channels were low in nutrients. Based on the rate of harvest and the quantity of nutrients in the plants (see TABLES 11 and 12) it is possible that excellent treatment occurred near the surface.

TABLE 8. RAINFALL DATA FOR TEST 1 STUDY PERIOD (CM)[a]

| March, 1976 |  | April, 1976 |  | May, 1976 |  |
|---|---|---|---|---|---|
| 3/8 | 1.52 | 4/3 | 0.33 | 5/8 | 2.97 |
| 3/14 | 1.50 | 4/25 | 1.09 |  |  |
| 3/15 | 0.46 | Total | 1.42 |  |  |
| 3/16 | 0.64 |  |  |  |  |
| 3/20 | 0.25 |  |  |  |  |
| 3/21 | 0.76 |  |  |  |  |
| 3/26 | 6.10 |  |  |  |  |
| 3/30 | 1.14 |  |  |  |  |
| 3/31 | 0.89 |  |  |  |  |
| Total | 12.34 |  |  |  |  |

---

[a] X .3937 for inches.

TABLE 9. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE IN mg/1 OF STATIC DAIRY WASTE LAGOONS WITH AND WITHOUT DUCKWEED (_S. oligorhiza_), SPRING, 1976 (TEST LAGOON 1)

| Date | Biochemical Oxygen Demand | | Chemical Oxygen Demand | | Total Residue | | Fixed Residue | | Volatile Residue | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C[a] | T | C | T | C | T | C | T | C | T |
| 4/8 | 385 | 475 | 1888 | 2096 | 1658 | 1586 | 698 | 590 | 960 | 996 |
| 4/22 | 334 | 480 | 1382 | 1512 | 2370 | 2182 | 1044 | 704 | 1326 | 1478 |
| 4/29 | 294 | 358 | 978 | 978 | 1740 | 1576 | 596 | 756 | 1144 | 820 |
| 5/6 | 188 | 269 | 891 | 979 | 1181 | 1229 | 474 | 346 | 707 | 883 |
| % reduction[b] | 51 | 43 | 53 | 53 | 29 | 23 | 32 | 41 | 26 | 11 |

[a] Control (C)
Test (T), average of 3 channels.

[b] Not corrected for evaporation.

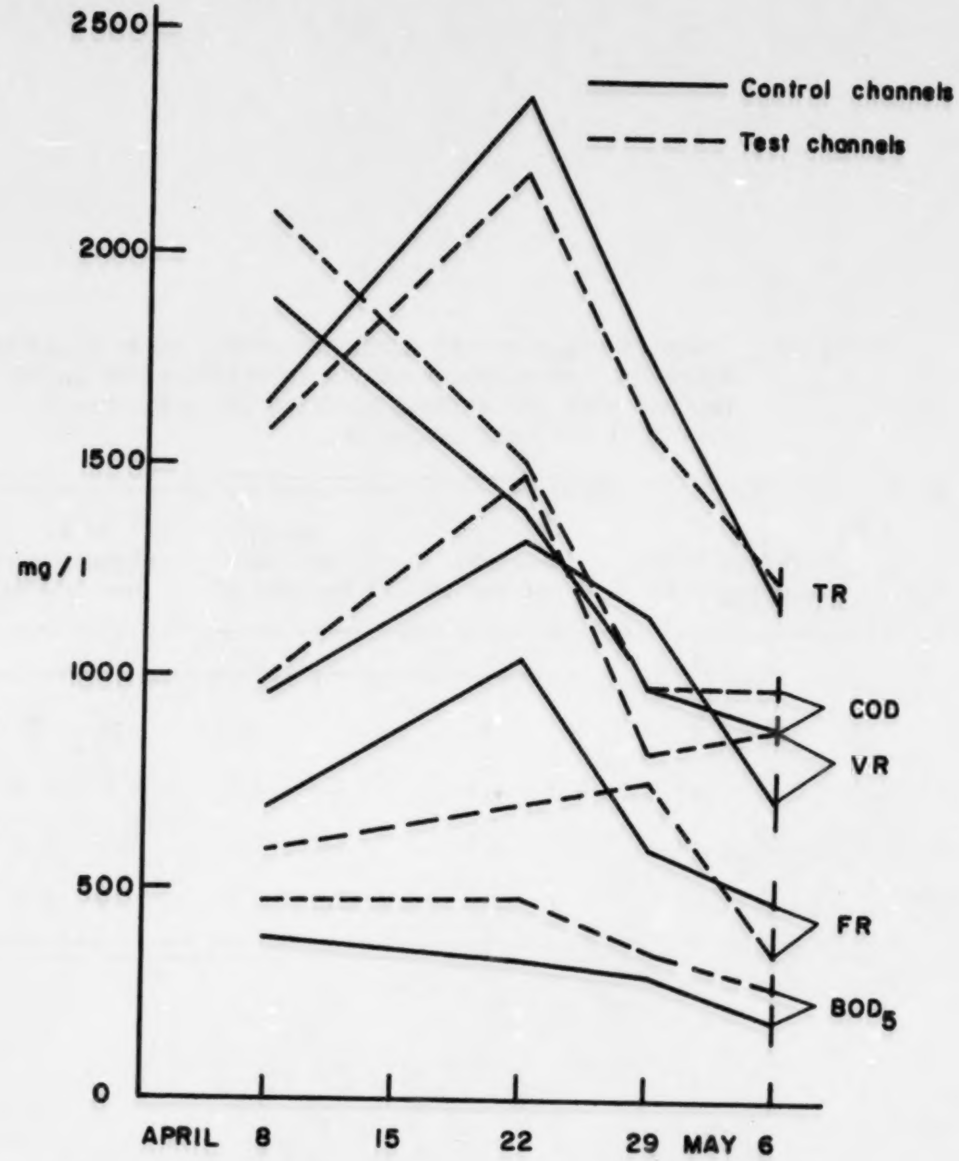

Figure 2. Biochemical oxygen demand ($BOD_5$), chemical oxygen demand (COD), total residue (TR), volatile residue (VR), and fixed residue (FR) in static dairy waste lagoon water with and without duckweed (S. oligorhiza), spring, 1976 (Test lagoon 1).

TABLE 10. STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS IN STATIC DAIRY WASTE LAGOONS WITH AND WITHOUT DUCKWEED (S. oligorhiza), SPRING, 1976 (TEST LAGOON 1)

| Date | Standard Plate Count per ml | | Anaerobic Count per ml | | Fecal Coliform per 100 ml | | Fecal Streptococci per 100 ml | |
|---|---|---|---|---|---|---|---|---|
| | x $10^6$ | | --------------------x $10^5$ | | | | ----------------------- | |
| | C | T | C | T | C | T | C | T |
| 4/22 | 2.1 | 2.5 | 4.3 | 4.5 | 3.3 | 3.1 | 4.9 | 1.8 |
| 4/29 | 2.1 | 2.3 | 5.7 | 5.1 | 2.3 | 2.3 | 3.3 | 1.1 |
| 5/6 | 2.5 | 1.4 | 3.9 | 3.3 | 3.3 | 3.3 | 3.3 | 2.3 |

TABLE 10. **STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS IN STATIC DAIRY WASTE LAGOONS WITH AND WITHOUT DUCKWEED** (S. oligorhiza), SPRING, 1976 (TEST LAGOON 1)

| Date | Standard Plate Count per ml | | Anaerobic Count per ml | | Fecal Coliform per 100 ml | | Fecal Streptococci per 100 ml | |
|---|---|---|---|---|---|---|---|---|
| | x $10^6$ | | --------------------x $10^5$---------------------- | | | | | |
| | C | T | C | T | C | T | C | T |
| 4/22 | 2.1 | 2.5 | 4.3 | 4.5 | 3.3 | 3.1 | 4.9 | 1.8 |
| 4/29 | 2.1 | 2.3 | 5.7 | 5.1 | 2.3 | 2.3 | 3.3 | 1.1 |
| 5/6 | 2.5 | 1.4 | 3.9 | 3.3 | 3.3 | 3.3 | 3.3 | 2.3 |

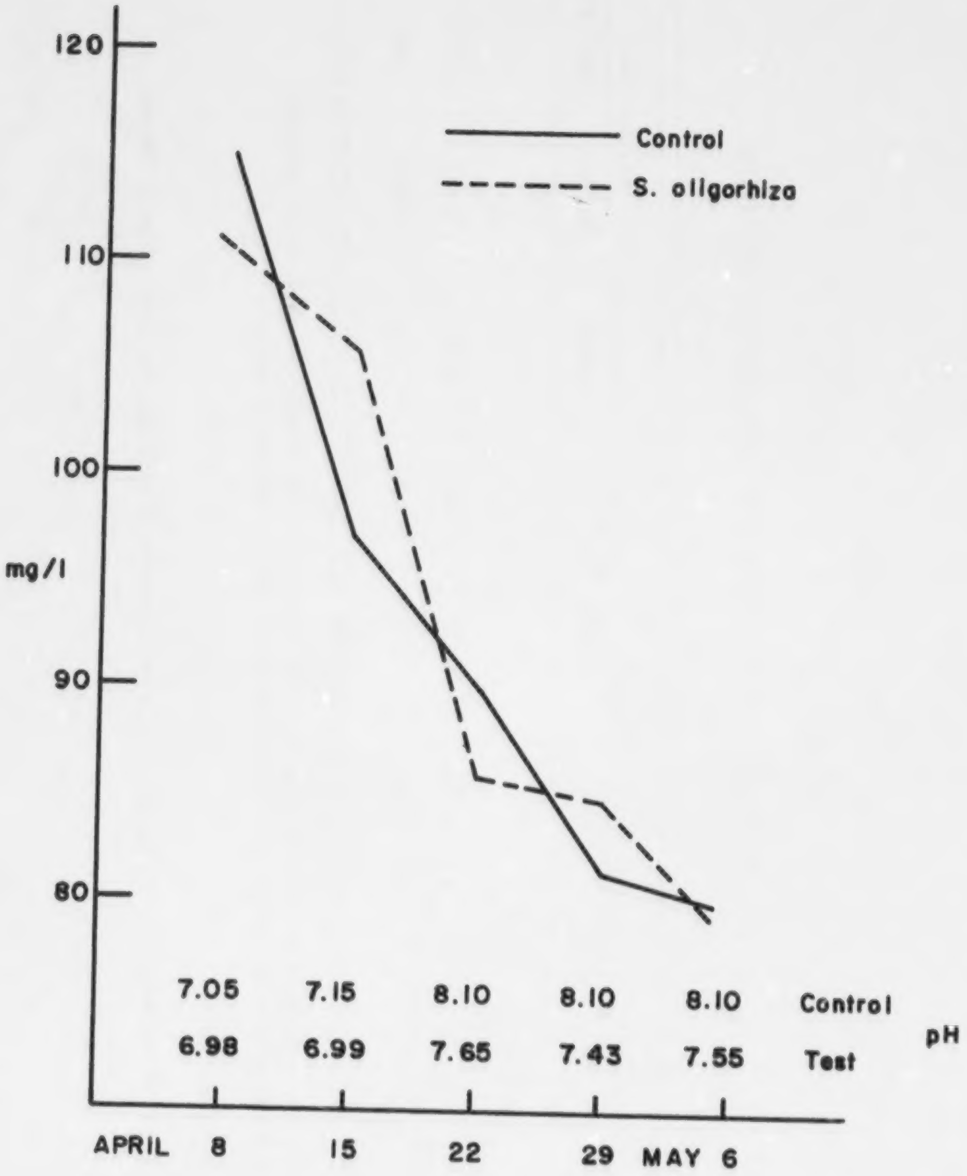

Figure 3. TKN reduction and pH in static dairy waste lagoons with and without stands of duckweeds, spring, 1976 (Test lagoon 1).

TABLE 11. *S. oligorhiza* BIOMASS HARVESTED[a] FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SPRING, 1976

| | Harvest date[b] | | | | Total/27 days | Harvest/mo[c] | |
|---|---|---|---|---|---|---|---|
| | 4/15 | 4/22 | 4/29 | 5/5 | | kg/ha | lbs/acre |
| kg harvested/$m^2$ | | | | | | | |
| wet | .66 | .485 | .389 | .487 | 2.02 | --- | --- |
| dry (9.2%) | .06 | .045 | .035 | .045 | .185 | 2056 | 1836 |

[a] Harvest data reflects actual biomass removed/$m^2$ from one-half (12.5 $m^2$) of each test channel. Only half would be harvested in order to leave duckweed stock for regrowth.

[b] Average of 4 test channels.

[c] Total harvest values in kg/ha/mo and lbs/acre/mo are based on one-half hectare or acre being harvested.

TABLE 12. PARTIAL CHEMICAL ANALYSIS OF *S. oligorhiza* GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SPRING, 1976

| Sample date | % moisture | % of dry matter[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | Fiber | Ash | Fats | TOC | Crude protein[b] |
| 4-9 | 92 | --- | --- | --- | --- | --- | |
| 4-13 | 90 | --- | --- | 6.8 | --- | --- | |
| 4-21 | 90 | 5.15 | 7.18 | 9.1 | --- | 30.5 | 32.19 |
| 4-29 | 90 | 4.92 | 8.28 | 7.0 | --- | 32.3 | 30.75 |
| 5-5 | 92 | 5.10 | 6.68 | 10.3 | --- | 36.3 | 31.88 |

[a] Average of four channels.

[b] Nitrogen x 6.25.

Undoubtedly fallout of organic matter accounted for some decline in the TKN, but how much is difficult to tell. Attempts to quantitatively measure the depth of the sludge layer were not successful as incoming suspended matter at the start of the test contributed an unknown amount of detritus to the channel bottom.

The TOC values were obtained for the lagoon water near the end of the study. The control channel TOC value was 210 and 200 mg/l for the last two sample periods, while the composited duckweed channels averaged 328 and 283 mg/l. The C:N ratio on May 5 was 2.5 to 1 for the control and 3.58 to 1 for the combined test channels for water sampled about 25 cm below the surface.

The test channels appeared to show higher buffering capacity than the control channels as the pH increased less (Figure 3) in the test channels. However, the duckweed cover undoubtedly reduced the incoming light, coupled with lower phytoplankton levels, the quantity of $CO_2$ produced could have been altered considerably.

TABLE 11 shows the kg wet duckweed harvested from one-half of each test channel at estimated doubling times for S. oligorhiza. The average dry biomass harvested/$m^2$ was .185 kg over the 27 day period. Projecting this figure to a hectare or acre basis, the total dry biomass that would have been harvested would be 2056 kg and 1851 lbs respectively. Based on nutrient analysis (TABLE 12) the duckweed averaged 31.6% crude protein, or 585 lbs of the biomass was protein. The low fiber and ash values shown in TABLE 12 show that the duckweed is a high quality feed material. The water content was low compared to later tests as will be seen. Duplication of drying showed these values to be accurate. Reasons for the high dry matter in this test are not known.

It is evident from TABLE 13 that protein values can be higher than reported in TABLE 12, and also in previous work where values exceeded 40% (Culley and Epps, 1973).

TABLE 13 shows growth of duckweed under continuous flow of effluent into test channels. These data were obtained in order to determine if the chemical content of duckweed was affected by a static vs. flowing system in which high nutrient levels could be maintained. Water and ash content of plants grown on the two systems were similar. Nitrogen, fiber, TOC, and crude protein gradually increased over time. It appears that a constant flowing system may improve duckweed feed value, but will also tend to complicate treatment as the retention time must be increased as waste treatment is offset by incoming effluent.

During this test water temperatures were recorded just under the water surface and 25 cm from the bottom. Surface temperatures ranged from 17° to 20°C and bottom temperatures from 16° to 18°C. Thermal stratification was evident in all channels. No temperature difference could be detected between test and control channels at the surface or bottom. Temperature differences between the surface and bottom ranged from .5 to 2.5°C. The

TABLE 13. PARTIAL CHEMICAL ANALYSIS OF *S. polyrhiza* GROWN ON TEST CHANNELS RECEIVING A CONTINUOUS FLOW OF DAIRY WASTE FROM THE STAGE 1 ANAEROBIC LAGOON, SPRING, 1976

| Sample date | % moisture | % of dry matter[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | Fiber | Ash | Fats | TOC | Crude protein[b] |
| 4-9 | 90 | 5.13 | 5.7 | 9.6 | --- | 34 | 32.06 |
| 4-13 | 90 | --- | --- | 8.0 | --- | --- | --- |
| 4-21 | --- | --- | --- | --- | --- | --- | --- |
| 4-29 | 91 | 5.40 | 9.8 | 5.8 | --- | 35 | 33.75 |
| 5-5 | 93 | 5.91 | 9.1 | 9.6 | --- | 44 | 36.94 |

[a] Average of six channels.

[b] Nitrogen x 6.25.

stage 1 anaerobic lagoon consistently had 2 to 5°C higher temperature and no vertical stratification.

This initial test indicated that the duckweed treatment performed equally to the control channels when the system received a high load of organic waste. The major difference indicated by harvesting the duckweed was that a considerable quantity of nutrients were removed by the duckweed whereas in the control channels many of these nutrients would remain in the bottom sediments or leave with the effluent.

### Test 2, Summer Treatment, 1976

The same basic procedures used in Test 1 were repeated here. Test lagoons 1 and 2 were flooded with the stage 1 anaerobic lagoon effluent, but at a lower nutrient level. Twelve channels were used, four each for S. oligorhiza, S. polyrhiza, and controls. Equal quantities of both plants were stocked (taken from previous test) on May 17 when the channels were reflooded. First harvest date was May 26 and was based on an average surface area of 25 $m^2$/channel. Water quality data was not corrected for evaporation, and collected about 12 cm below the surface. Productivity and nutrient content was again determined for the duckweeds.

Only washwater from the holding pens and milking parlor was allowed to enter the stage 1 anaerobic lagoon. Data on the lagoon are presented in TABLES 14, 15, and 16. A crust formed on the lagoon before the study was initiated and the continual build up and breakdown of this crust along with daily loading undoubtedly caused variation in the outlet sample. The general increase in bacterial counts (TABLE 16) is due to increasing temperature and continued high nutrient conditions. TABLES 18 through 21 and Figures 4 through 11 show water quality changes during this study and TABLE 17 shows rainfall. Water quality values were not corrected for evaporation. Although nearly 17 cm of rain occurred during the 50 days, evaporation still exceeded rainfall. Water quality values would have been about 20% lower had evaporation equaled rainfall.

It is clearly evident from all water quality values recorded in TABLES 18 through 21 that the duckweed-covered channels performed as well as the control channels in water quality renovation. Although there was considerable fluctuation on specific sampling days for several parameters, by the end of the test most values were similar, and the actual value change for each parameter was similar.

TABLES 22 through 25 and Figures 12 through 14 show surface water bacterial population changes with treatment. Responses again show test and control channels performed similarly in bacterial reduction. Bacteria counts were lower in two of the four categories at the end of the test, but the differences were slight. It is pertinent to point out again, that results obtained should not be taken as expected values under a well managed system in which large quantities of insoluble matter would not enter duckweed-covered lagoons. TABLE 26 shows the average percent reduction of chemical and microbial parameters for combined channels. Calcium was not

TABLE 14. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, AND FIXED RESIDUE OF THE STAGE 1 ANAEROBIC DAIRY LAGOON EFFLUENT

| Date | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue | Total Suspended Matter |
|---|---|---|---|---|---|---|
| | mg/1 | | | | | |
| 5/17/76 | 498 | 2149 | 2382 | 1642 | 740 | 800 |
| 5/26 | 317 | 1984 | 2116 | 1376 | 740 | 400 |
| 6/4 | 256 | 1052 | 1864 | 1290 | 874 | --- |
| 6/14 | --- | 1767 | 2044 | 1350 | 694 | 770 |
| 6/18 | --- | 2450 | 3148 | 1576 | 1572 | 2140 |
| 6/24 | --- | 1656 | 2570 | 1540 | 1030 | 1160 |
| 7/1 | 495 | 1842 | 2434 | 1932 | 502 | 1140 |
| Average Value | 392 | 1843 | 2365 | 1529 | 879 | 1068 |

TABLE 15. STAGE 1 ANAEROBIC LAGOON WATER QUALITY[a]

| Date | TKN(mg/l) | K(mg/l) | Ca(mg/l) | pH | Temperature(°C) |
|---|---|---|---|---|---|
| 5/27 | 89.9 | 97.7 | 54.6 | 6.80 | 24.4 |
| 5/31 | 105.9 | 97.7 | 50.4 | 6.85 | 25.0 |
| 6/6 | 112.4 | 102.0 | 43.1 | 7.02 | 25.6 |
| 6/14 | 90.8 | 101.3 | 38.0 | 6.61 | 26.7 |
| 6/18 | 116.2 | --- | --- | 6.84 | 27.2 |
| 6/24 | 117.5 | 74.3 | 64.1 | 6.98 | 27.8 |
| 7/1 | 128.1 | --- | --- | 6.85 | 27.8 |
| 7/8 | 126.7 | 150.8 | 67.5 | 6.61 | 28.3 |
| 7/15 | 125.9 | 138.1 | 69.9 | 6.75 | --- |
| 7/22 | 104.3 | 152.8 | 56.9 | 6.85 | --- |
| 7/29 | 111.7 | 141.4 | 60.1 | 6.68 | 26.7 |
| 8/5 | 101.9 | 143.0 | 55.4 | 6.86 | 26.7 |
| 9/1 | 69.2 | 84.5 | 45.5 | 6.62 | --- |
| 9/8 | 80.4 | 79.6 | 43.9 | 6.65 | --- |

[a] Received only wash water from the loafing area and milking parlor. Considerable manure loaded in February and March was still present.

TABLE 16. STANDARD PLATE COUNT, ANAEROBIC COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE STAGE 1 ANAEROBIC LAGOON EFFLUENT

| Date | Standard Plate Count per ml | Anaerobic Count per ml | Fecal Coliform per 100 ml | Fecal Streptococci per 100 ml |
|---|---|---|---|---|
| | x $10^6$ | | | |
| 5/17/76 | 3.2 | 2.2 | 1.8 | 1.3 |
| 5/26 | 2.5 | 2.0 | 2.2 | 2.0 |
| 6/4 | 2.6 | 5.5 | 2.8 | 2.0 |
| 6/14 | 4.9 | 3.9 | 4.7 | 3.8 |
| 6/18 | 2.7 | 4.4 | 4.8 | 4.1 |
| 6/24 | 2.1 | 3.1 | 5.1 | 4.8 |
| 7/1 | 3.2 | 3.0 | 4.1 | 3.7 |
| Average Value | 3.0 | 3.4 | 3.6 | 3.1 |

TABLE 17. RAINFALL DATA FOR TEST 2 STUDY PERIOD[a] (CM)[b]

| May 1976 | | June 1976 | | July 1976 | |
|---|---|---|---|---|---|
| 5/25 | 0.89 | 6/1 | 1.91 | 7/1 | 1.37 |
| 5/28 | 1.40 | 6/5 | 1.14 | 7/5 | 4.01 |
| Total | 2.29 | 6/19 | 1.60 | 7/17 | 3.12 |
| | | 6/29 | 5.21 | 7/20 | 2.49 |
| | | 6/30 | 0.15 | 7/23 | 1.09 |
| | | Total | 10.01 | 7/24 | 2.21 |
| | | | | 7/25 | 2.62 |
| | | | | 7/26 | 0.13 |
| | | | | Total | 17.04 |

[a] Study initiated May 17 and terminated July 15.

[b] X .3937 for inches.

TABLE 18. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF THE *S. oligorhiza* TEST CHANNELS, SUMMER, 1976

| Date | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue | Total Suspended Matter |
|---|---|---|---|---|---|---|
| | | | mg/l | | | |
| 5/17/76 | 278 | 782 | 934 | 643 | 291 | 620 |
| 5/26 | 91 | 732 | 1078 | 908 | 170 | 300 |
| 6/4 | 47 | 341 | 724 | 386 | 338 | --- |
| 6/14 | --- | 293 | 922 | 552 | 370 | 100 |
| 6/18 | --- | 304 | 742 | 400 | 342 | 86 |
| 6/24 | --- | 266 | 892 | 645 | 247 | 84 |
| 7/1 | 20 | 217 | 735 | 436 | 299 | 94 |
| 7/8 | --- | 279 | 668 | 442 | 226 | 108 |
| 7/15 | --- | 180 | 382 | 198 | 184 | 93 |

TABLE 19. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF COMPOSITE SAMPLE OF THE S. *polyrhiza* TEST CHANNELS, SUMMER, 1976

| Date | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue | Total Suspended Matter |
|---|---|---|---|---|---|---|
| | ----------- | ----------- | ----------- | mg/l | ----------- | ----------- |
| 5/17/76 | 236 | 807 | 1087 | 743 | 344 | 690 |
| 5/26 | 60 | 516 | 980 | 718 | 262 | 330 |
| 6/4 | 51 | 403 | 794 | 432 | 362 | --- |
| 6/14 | --- | 301 | 1078 | 590 | 488 | 170 |
| 6/18 | --- | 316 | 714 | 392 | 322 | 114 |
| 6/24 | --- | 216 | 939 | 735 | 204 | 108 |
| 7/1 | 18 | 202 | 683 | 388 | 295 | 104 |
| 7/8 | --- | 271 | 584 | 358 | 226 | 109 |
| 7/15 | --- | 184 | 596 | 460 | 136 | 91 |

TABLE 20. BIOCHEMICAL OXYGEN DEMAND, CHEMICAL OXYGEN DEMAND, TOTAL RESIDUE, VOLATILE RESIDUE, FIXED RESIDUE, AND TOTAL SUSPENDED MATTER OF THE COMPOSITE SAMPLE FROM THE CONTROL CHANNELS, SUMMER, 1976

| Date | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue | Total Suspended Matter |
|---|---|---|---|---|---|---|
| | mg/l | | | | | |
| 5/17/76 | 248 | 861 | 938 | 600 | 338 | 630 |
| 5/26 | 52 | 616 | 893 | 597 | 296 | 320 |
| 6/4 | 33 | 263 | 692 | 472 | 220 | --- |
| 6/14 | --- | 274 | 790 | 516 | 274 | 110 |
| 6/18 | --- | 336 | 773 | 450 | 323 | 122 |
| 6/24 | --- | 230 | 857 | 587 | 270 | 104 |
| 7/1 | 17 | 209 | 769 | 489 | 280 | 104 |
| 7/8 | --- | 271 | 722 | 484 | 238 | 111 |
| 7/15 | --- | 209 | 550 | 352 | 198 | 101 |

TABLE 21. TKN, POTASSIUM, CALCIUM, TOC, pH AND WATER TEMPERATURE OF TEST AND CONTROL CHANNELS[a] SUMMER, 1976

| Date | TKN(mg/l) | | | K(mg/l) | | | Ca(mg/l) | | | TOC(mg/l) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [b]S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C |
| 5/17/76 | 82.2 | 75.0 | 76.8 | --- | --- | --- | --- | --- | --- | --- | --- | --- | 7.1 | 7.3 | 7.4 |
| 5/27 | 69.6 | 60.1 | 63.8 | 91.1 | 92.7 | 94.3 | 63.0 | 63.0 | 64.2 | 155 | 125 | 120 | 7.6 | 7.8 | 8.0 |
| 5/31 | 63.9 | 64.0 | 61.7 | 88.9 | 90.1 | 96.0 | 60.3 | 62.0 | 64.7 | 120 | 150 | 135 | 7.6 | 7.7 | 8.0 |
| 6/6 | 59.9 | 58.9 | 59.4 | 90.3 | 91.9 | 93.6 | 66.4 | 65.5 | 69.3 | 225 | 220 | 225 | 7.7 | 7.6 | 8.0 |
| 6/14 | 53.5 | 54.7 | 53.1 | 91.4 | 94.0 | 96.9 | 59.4 | 62.4 | 64.0 | --- | --- | --- | 7.5 | 7.3 | 7.8 |
| 6/18 | 50.1 | 53.3 | 49.7 | 106.6 | 103.5 | 108.8 | 61.9 | 60.9 | 61.0 | --- | --- | --- | 7.7 | 7.5 | 8.0 |
| 6/24 | 43.8 | 47.0 | 45.1 | 96.0 | 95.4 | 96.8 | 77.7 | 77.4 | 86.0 | 110 | 98 | 99 | 7.9 | 7.7 | 7.9 |
| 7/1 | 34.4 | 33.9 | 36.5 | --- | --- | --- | --- | --- | --- | 84 | 81 | 85 | 7.7 | 7.7 | 7.8 |
| 7/8[c] | 24.0 | 26.0 | 28.5 | 80.1 | 84.4 | 93.4 | 56.3 | 61.3 | 68.1 | 83 | 84 | 85 | 7.7 | 7.5 | 8.1 |
| 7/15 | 23.3 | 17.1 | 20.7 | 75.4 | 72.6 | 81.0 | 55.1 | 60.2 | 58.3 | --- | --- | --- | 7.8 | 7.6 | 7.8 |

(Continued).

TABLE 21 (continued).

| Date | Water temperature | | | |
|---|---|---|---|---|
| | S.o. and S.p. | | Control | |
| | Top | Bottom | Top | Bottom |
| 5/17 | 20.2 | 19.5 | 21.4 | 19.5 |
| 5/27 | 21.3 | 19.1 | 21.5 | 19.5 |
| 5/31 | 22.8 | 19.3 | 25.0 | 19.4 |
| 6/6 | 24.0 | 20.2 | 25.5 | 20.3 |
| 6/14 | 25.0 | 20.7 | 25.9 | 20.6 |
| 6/18 | 25.9 | 21.6 | 26.3 | 21.4 |
| 6/24 | 26.7 | 21.9 | 26.9 | 21.8 |
| 7/2 | 27.4 | 22.2 | 27.4 | 22.2 |
| 7/8 | 27.5 | 22.3 | 27.4 | 22.2 |
| 7/29 | 26.8 | 25.9 | --- | --- |
| 8/5 | 26.9 | 25.8 | --- | --- |

[a] Average of 4 channels.

[b] S.o. (*Spirodela oligorhiza*), S.p. (*Spirodela polyrhiza*), C (control).

[c] Average of 2 channels beginning on 7/8.

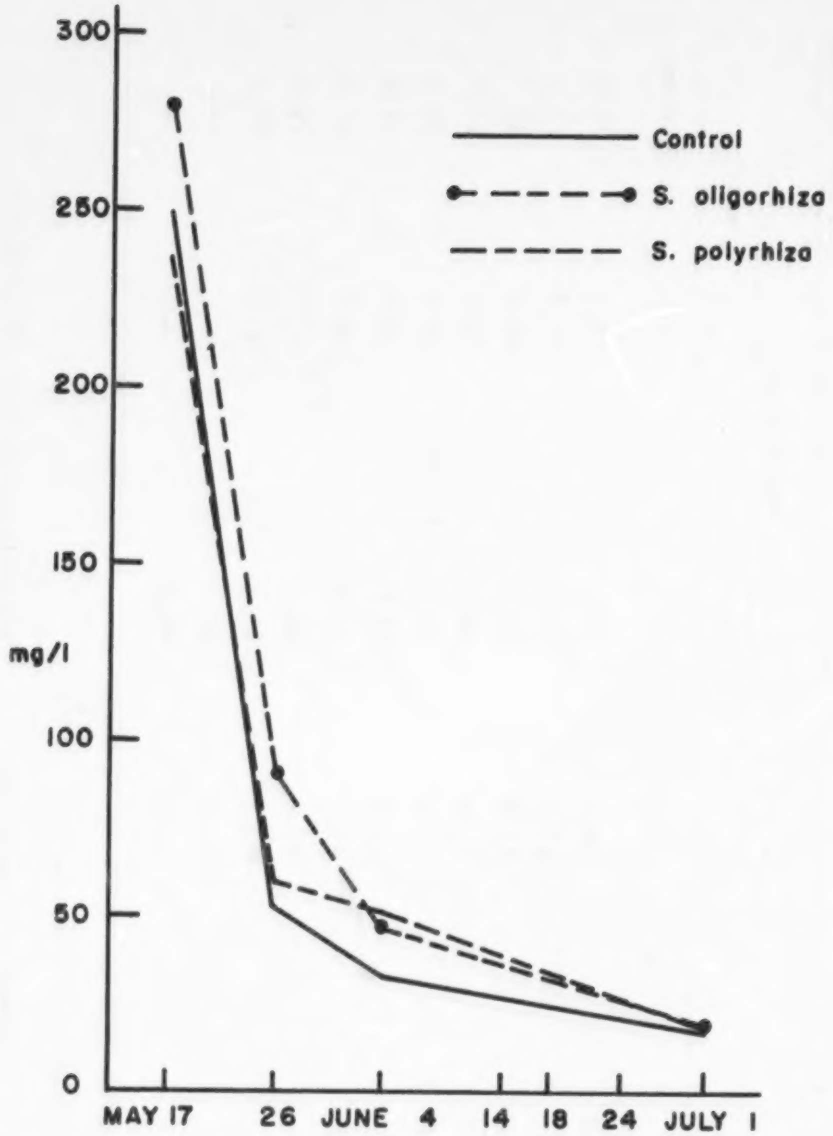

Figure 4. 5-day biochemical oxygen demand in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

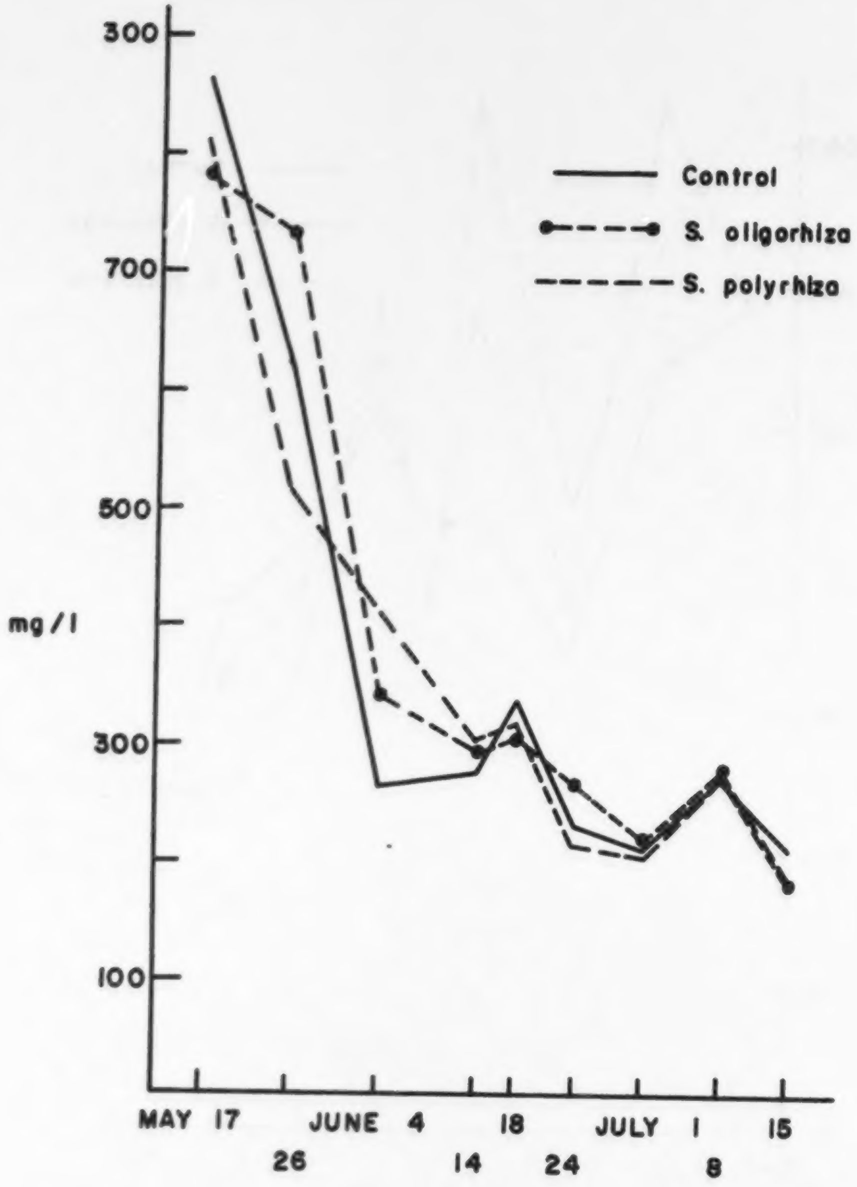

Figure 5. Chemical oxygen demand in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

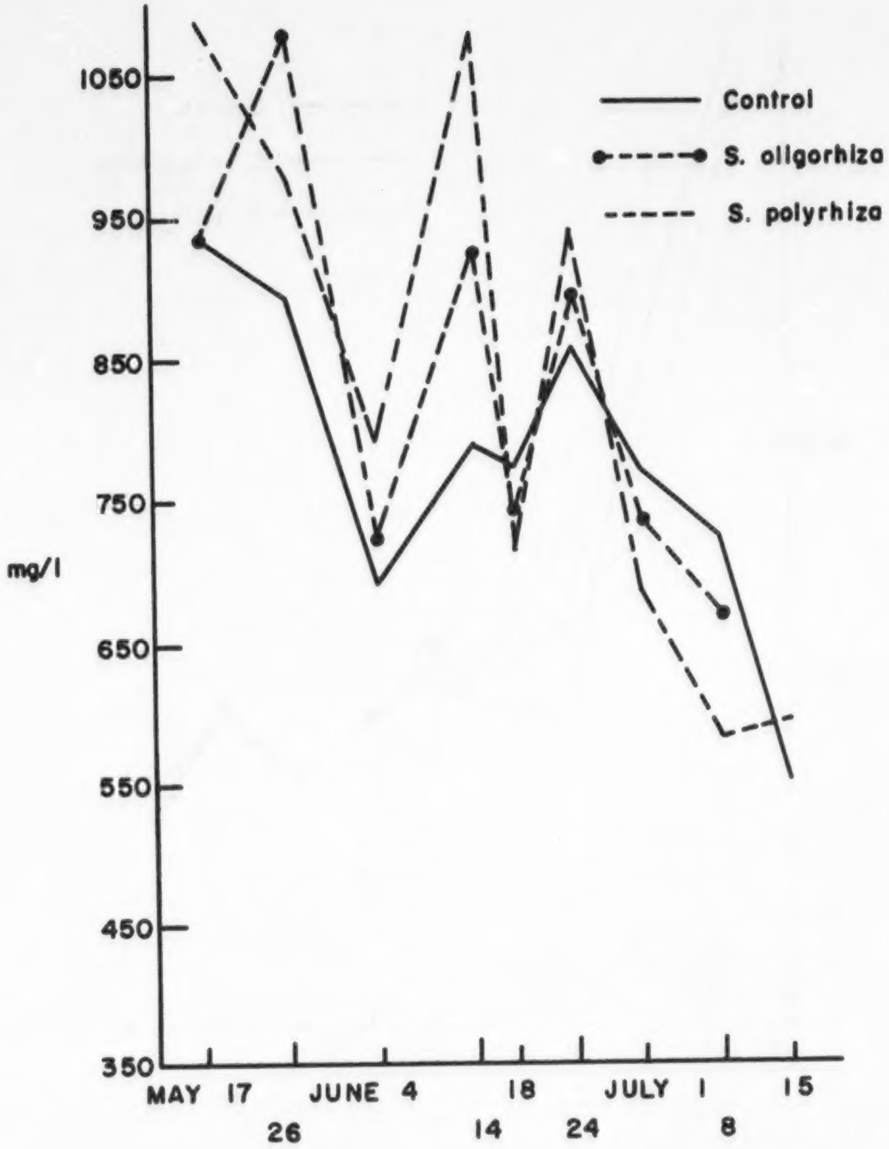

Figure 6. Total residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

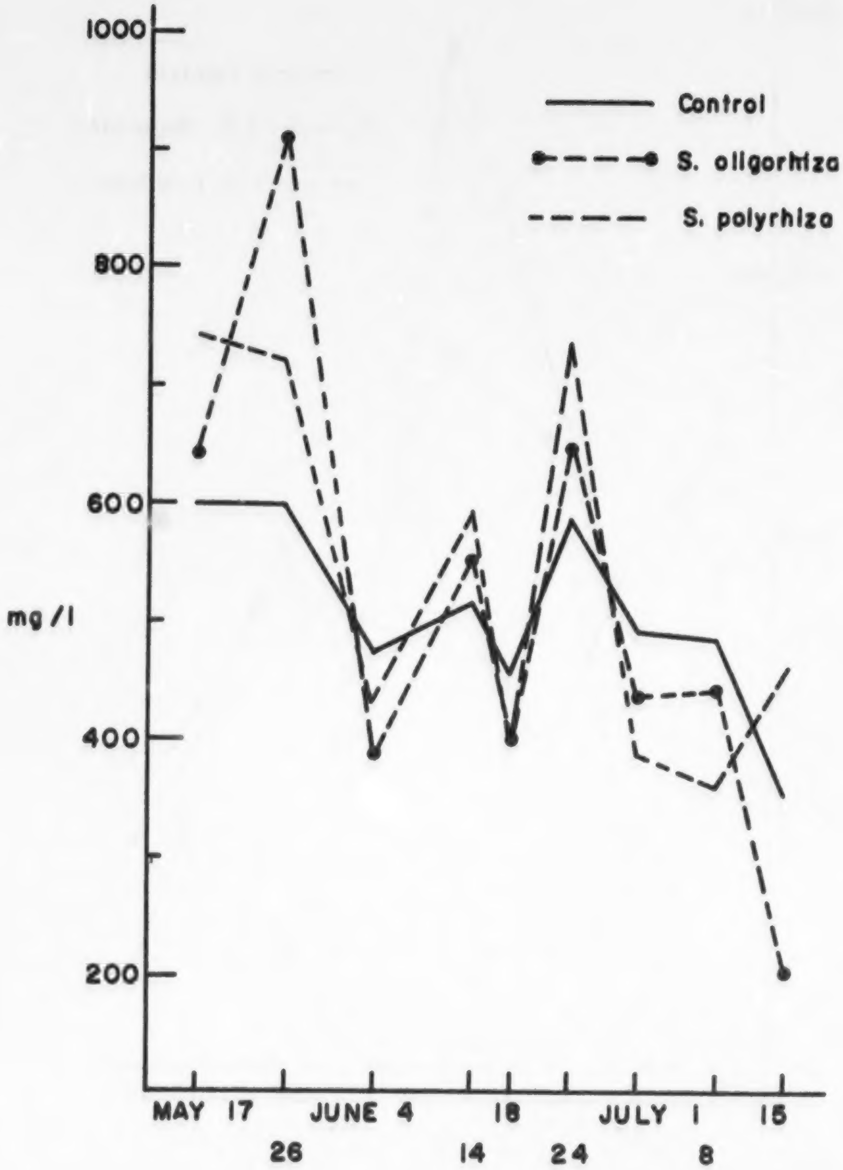

Figure 7. Volatile residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

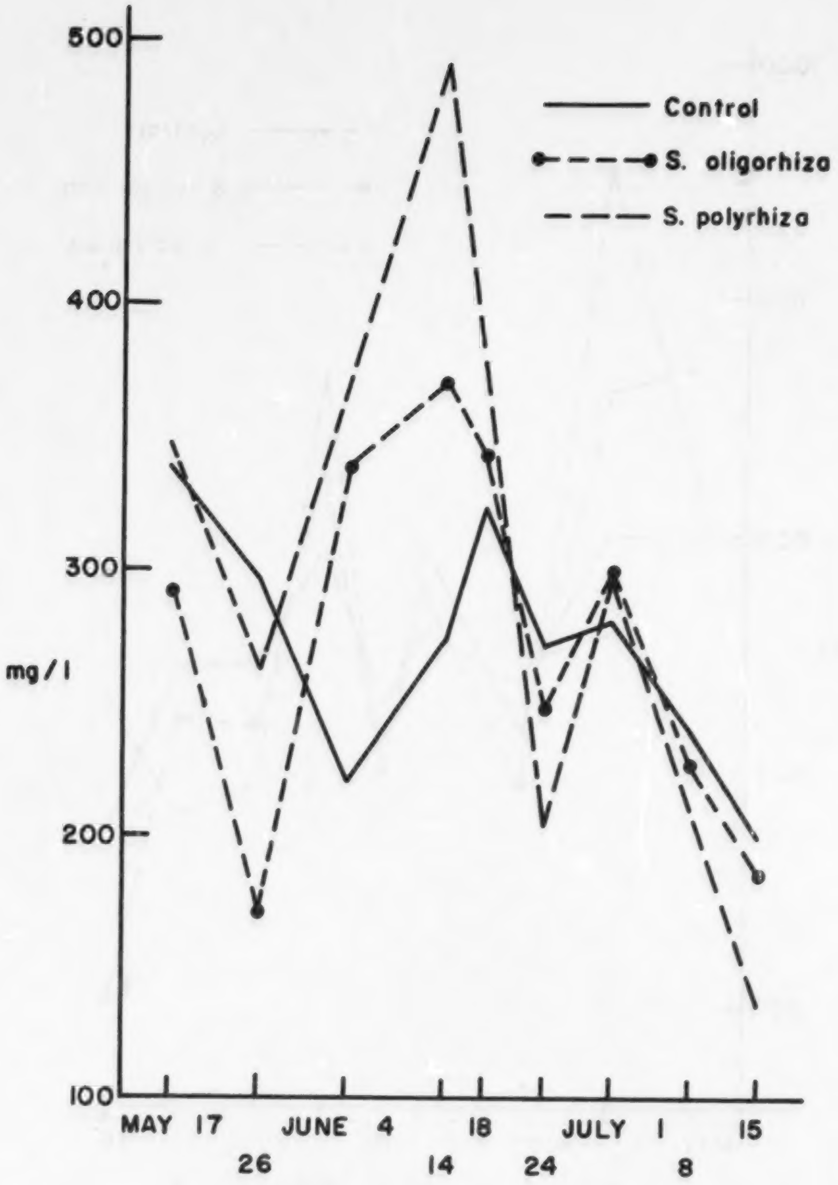

Figure 8. Fixed residue in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

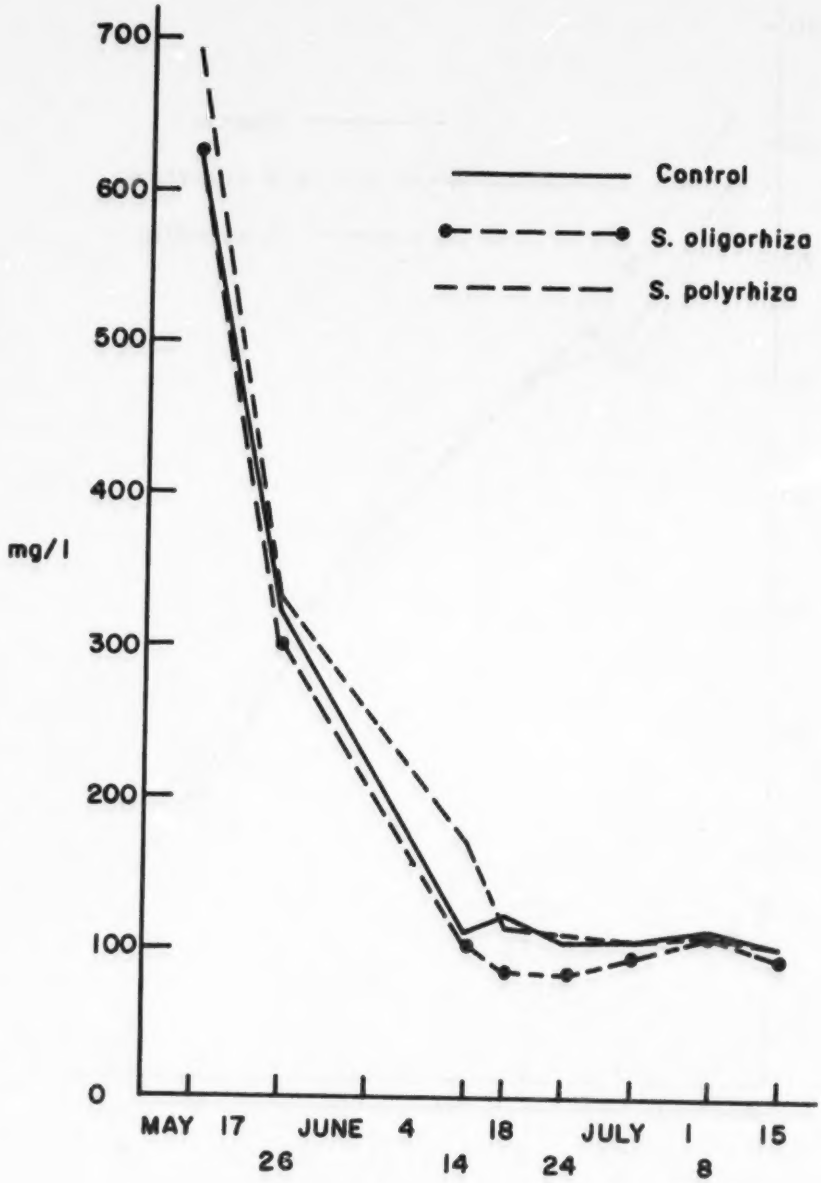

Figure 9. Total suspended matter in static dairy waste lagoons with and without stands of duckweeds, summer, 1976.

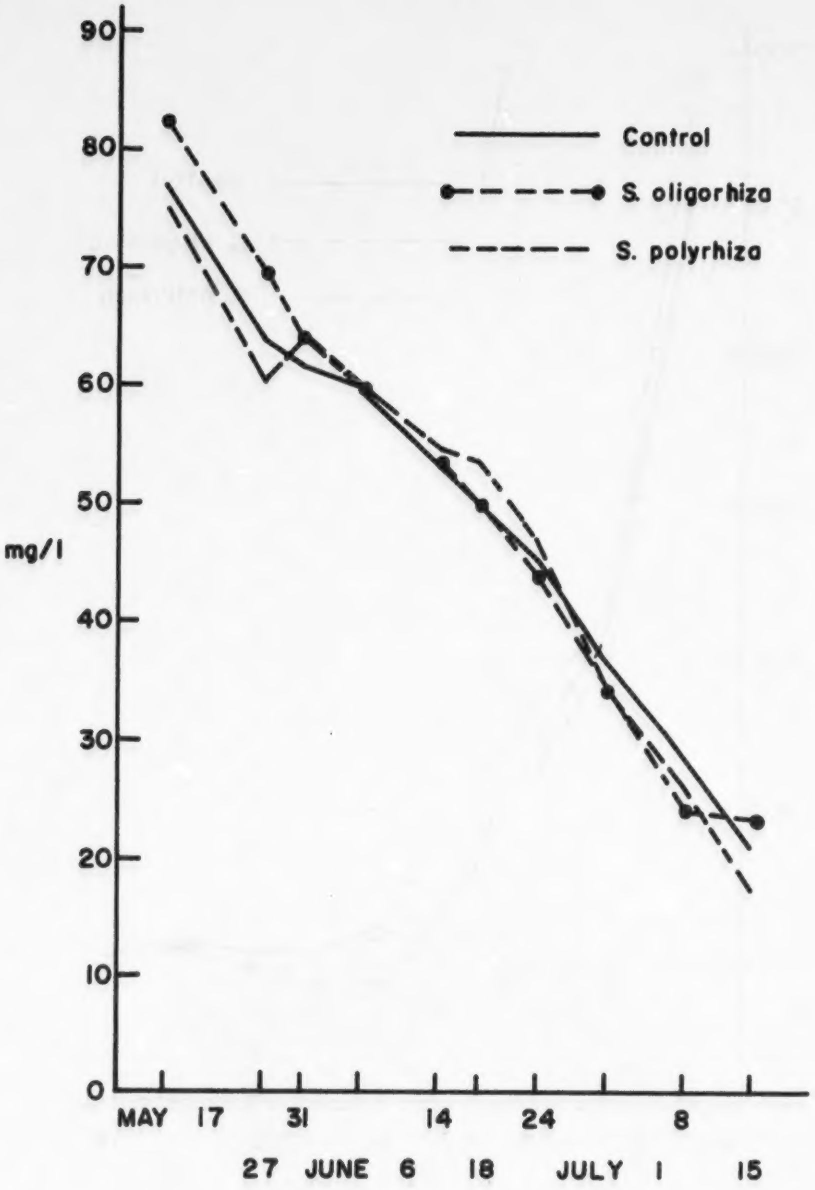

Figure 10. TKN reduction in test channels covered with duckweed, and controls, summer, 1976.

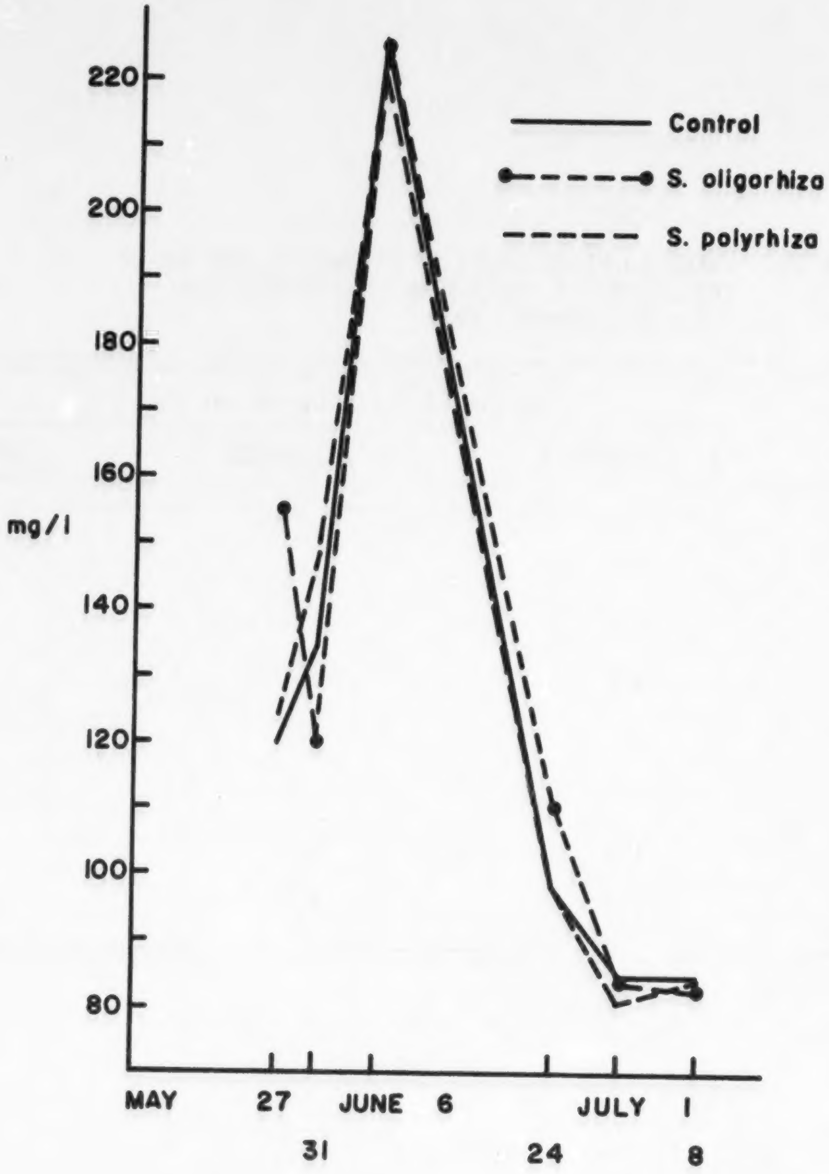

Figure 11. TOC reduction in test channels covered with duckweed, and controls, summer, 1976.

TABLE 22. STANDARD PLATE COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976

| Date | Standard Plate Count per ml | | |
|---|---|---|---|
| | *S. oligorhiza* | *S. polyrhiza* | Control |
| | --------------------------x $10^6$ | -------------------------- | |
| 5/17/76 | 3.0 | 2.6 | 2.5 |
| 5/26 | 2.5 | 1.7 | 1.5 |
| 6/4 | 4.9 | 4.6 | 1.7 |
| 6/14 | 1.7 | 1.1 | 1.9 |
| 6/18 | 1.3 | 1.6 | 1.7 |
| 6/24 | 1.5 | 1.3 | 1.3 |
| 7/1 | 2.3 | 1.6 | 1.9 |

TABLE 23. ANAEROBIC COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976

| Date | Anaerobic Count per ml | | |
|---|---|---|---|
| | *S. oligorhiza* | *S. polyrhiza* | Control |
| | x $10^5$ | | |
| 5/17/76 | 3.3 | 3.8 | 3.4 |
| 5/26 | 3.1 | 3.1 | 3.3 |
| 6/4 | 4.8 | 3.3 | 4.6 |
| 6/14 | 4.6 | 5.3 | 4.1 |
| 6/18 | 1.2 | 1.8 | 1.5 |
| 6/24 | 1.4 | 1.2 | 1.5 |
| 7/1 | 1.4 | 1.7 | 1.4 |

TABLE 24. FECAL COLIFORM COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976

| Date | Fecal Coliform per 100 ml | | |
|---|---|---|---|
| | *S. oligorhiza* | *S. polyrhiza* | Control |
| | ------------------------x $10^5$ | --------------------- | |
| 5/17/76 | 2.0 | 2.5 | 2.1 |
| 5/26 | 3.3 | 3.0 | 2.4 |
| 6/4 | 3.5 | 5.0 | 3.4 |
| 6/14 | 3.6 | 4.4 | 1.4 |
| 6/18 | 3.7 | 4.1 | 2.0 |
| 6/24 | 2.3 | 2.7 | 2.3 |
| 7/1 | 1.9 | 1.6 | 1.2 |

TABLE 25. FECAL STREPTOCOCCI COUNT OF COMPOSITE SAMPLES OF TEST CHANNELS COVERED WITH DUCKWEED, AND CONTROLS, SUMMER, 1976

| | Fecal Streptococci per 100 ml | | |
|---|---|---|---|
| Date | *S. oligorhiza* | *S. polyrhiza* | Control |
| | x $10^5$ | | |
| 5/17/76 | 1.7 | 1.8 | 1.5 |
| 5/26 | 1.9 | 2.6 | 1.4 |
| 6/4 | 2.1 | 2.3 | 2.0 |
| 6/14 | 2.1 | 3.0 | 1.0 |
| 6/18 | 2.0 | 2.6 | 1.1 |
| 6/24 | 2.4 | 2.6 | 2.2 |
| 7/1 | 1.6 | 1.4 | 0.9 |

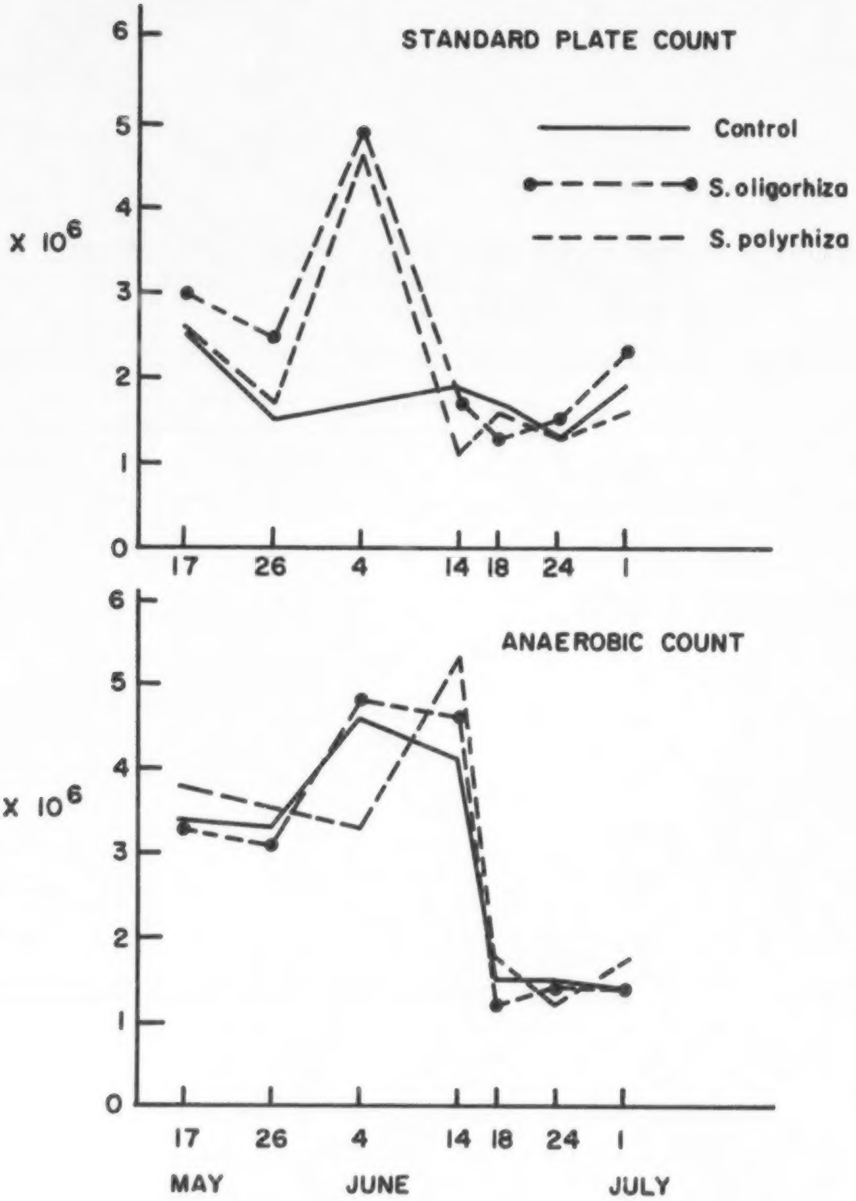

Figure 12. Standard bacteria plate count and anaerobic counts per ml of composite samples of the test channels covered with duckweed, and controls, summer, 1976.

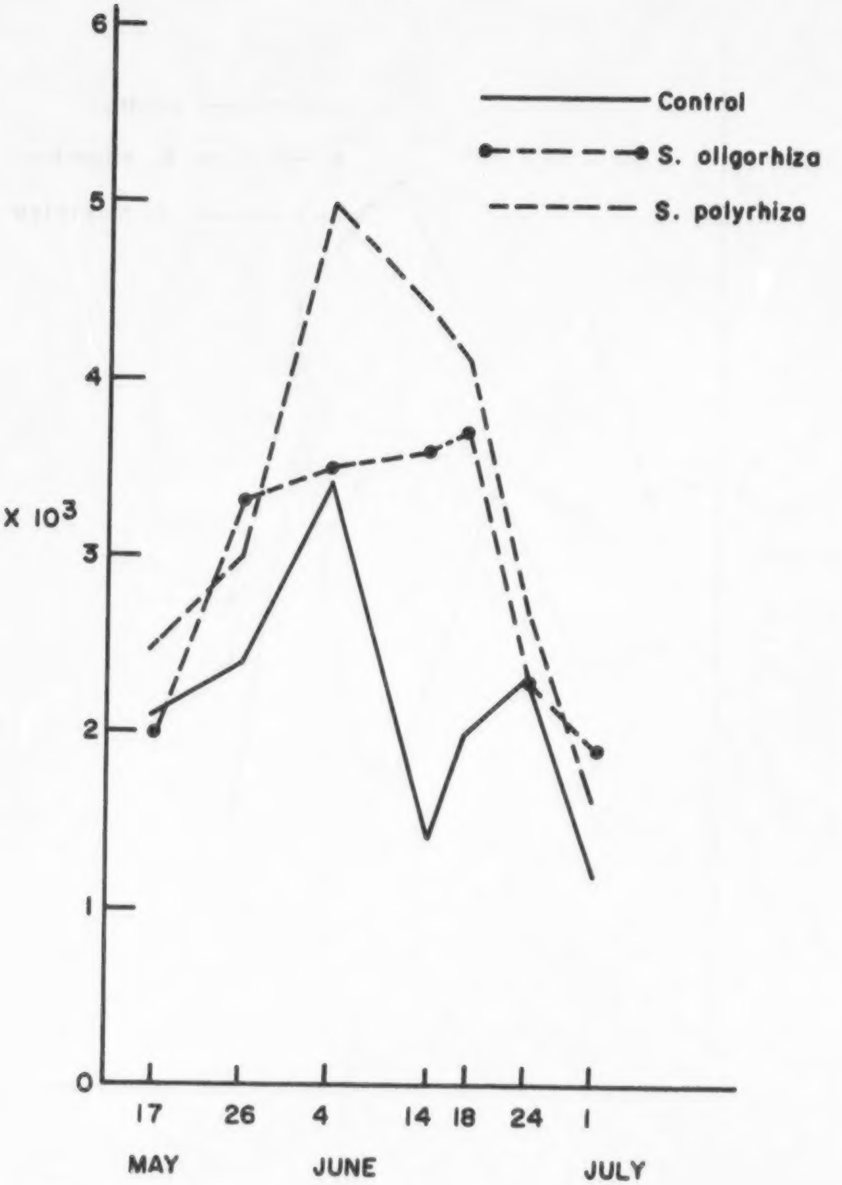

Figure 13. Fecal coliform per ml of composite samples of test channels covered with duckweed, and controls, summer, 1976.

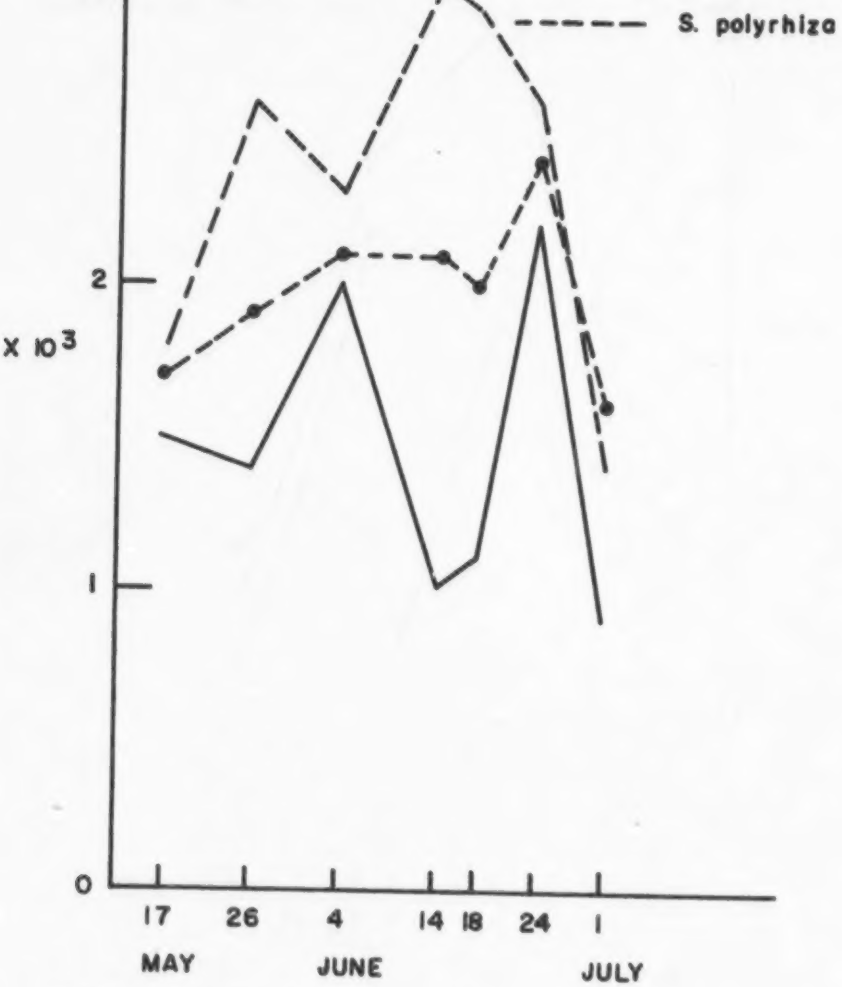

Figure 14. Fecal streptococci count per ml of composite samples of test channels covered with duckweed, and controls, summer, 1976.

TABLE 26. PERCENT REDUCTION IN TEST AND CONTROL CHANNEL WATER QUALITY AND BACTERIAL COUNTS, SUMMER, 1976

| | Biochemical Oxygen Demand | Chemical Oxygen Demand | Total Residue | Volatile Residue | Fixed Residue | Total Suspended Matter | Total Kjeldahl Nitrogen |
|---|---|---|---|---|---|---|---|
| *S. oligorhiza* | 92 | 77 | 59 | 69 | 37 | 85 | 72 |
| *S. polyrhiza* | 93 | 77 | 45 | 38 | 60 | 87 | 77 |
| Controls | 93 | 76 | 41 | 41 | 41 | 84 | 73 |

| | Potassium | Total Organic Carbon | Standard Plate Count | Anaerobic Count | Fecal Coliform Count | Fecal Streptococci |
|---|---|---|---|---|---|---|
| *S. oligorhiza* | 18 | 46 | 23 | 58 | 5 | 6 |
| *S. polyrhiza* | 22 | 33 | 38 | 55 | 36 | 22 |
| Controls | 14 | 29 | 24 | 59 | 43 | 40 |

included as a decline was not clearly evident. Potassium did show a little decline. Both of these elements were not expected to decline because of their abundance in the soil.

The percent decline in bacterial counts were low. The values were calculated from the counts on the first and last day. However, the counts increased up to the third to fifth sampling period and then declined. Percent decline for the fecal coliform under S. oligorhiza, S. polyrhiza, and control would have been 49%, 68%, and 65% respectively had the highest count been used. Similar increases in the percent decline were evident in other bacterial counts. High copepod populations developed as the bacterial counts peaked. They may have contributed significantly to the bacterial decline as they feed on the bacteria.

In the control channels the pH drifted up to a value of 8, while in the test channels it remained between 7.5 to 7.7. As the water quality improved in all channels, the pH stabilized between 7.6 to 7.8. The higher values in the control probably related to photosynthetic activity by phytoplankton in the water column. As the nutrients became depleted phytoplankton biomass decreased as did the photosynthetic rate, and therefore the pH.

During the 59 days of the test TKN reduction averaged 1 mg/l/day, not taking into account that evaporation served to concentrate TKN. This daily redution is less than the TKN reduction observed in Test 1 where higher nutrients were recorded.

In developing a management scheme for lagoons, with or without duckweed, nutrient loading where TKN is less than 100 mg/l may be more efficient in achieving acceptable water quality as the total percent reduction was greater at the lower level. Further analysis is needed however, because the sludge layer on the lagoon floor may have contributed to the nutrients in the water column. Because in the initial study and Test 2, the lagoons were flooded with an effluent high in nutrients and suspended matter, a nutrient reservoir developed on the bottom. Under a controlled system, flow of soluble nutrients into the aquatic plant lagoons should be maximized, and flow of suspended organic matter minimized. In an effort to evaluate the effect of nutrient-laden sludge on the nutrients in the water column, a test was set up to resuspend the sludge by mixing. This study (Test 3) is discussed in the next section.

The growth response and chemical content of the two duckweeds are shown in TABLES 27, 28, 29, and 30. Compared to Test 1 (spring) S. oligorhiza had reduced growth. S. polyrhiza developed rapidly and showed excellent growth. It is not known if our clone of S. oligorhiza was affected by the increased temperature, daylength, or nutrients. Previous to this project both species had grown well under cool temperatures and a shorter daylength. However, in the spring test the S. polyrhiza clone developed slowly, but grew rapidly as summer conditions appeared. Factors other than genetics and nutrients complicate the growth of duckweeds and this study was not designed to determine these various factors. For example, duckweeds are known to show periodicity in growth unrelated to temperature. With this knowledge, management schemes should give proper consideration to utilizing mixed

TABLE 27. *S. oligorhiza* BIOMASS HARVESTED[a] FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| | Harvest date[b] | | | | | | | | Total/59 | Harvest/mo[c] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5/26 | 6/6 | 6/14 | 6/18 | 6/24 | 7/1 | 7/8 | 7/15 | days | kg/ha | lbs/acre |
| kg harvested/$m^2$ | | | | | | | | | | | |
| wet | .51 | .26 | .45 | .50 | .29 | .43 | .36 | .41 | 3.21 | --- | --- |
| dry (7.96%) | .041 | .021 | .036 | .040 | .023 | .034 | .029 | .033 | 0.257 | 1307 | 1167 |

[a] Values show the average amount removed (½) from a $m^2$.

[b] Average of 4 test channels except for 7/8 and 7/15 which are the average of 2 channels.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

TABLE 28. *S. polyrhiza* BIOMASS HARVESTED[a] FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| | Harvest date[b] | | | | | | | | Total/59 | Harvest/mo[c] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5/26 | 6/6 | 6/14 | 6/18 | 6/24 | 7/1 | 7/8 | 7/15 | days | kg/ha | lbs/acre |
| kg harvested/$m^2$ | | | | | | | | | | | |
| wet | --- | .82 | .73 | .64 | .50 | .52 | .50 | .79 | 4.50 | --- | --- |
| dry | --- | .048 | .043 | .038 | .030 | .031 | .030 | .047 | .267 | 1358 | 1213 |

[a] Values show the average biomass removed (½) from a $m^2$.

[b] Average of 4 test channels except for 7/8 and 7/15 which are averages of 2 channels.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

### TABLE 29. PARTIAL CHEMICAL ANALYSIS OF *S. oligorhiza* GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| Sample Date | % Moisture | % of dry matter[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | Fiber | Ash | TOC | Silica | Crude Protein[b] |
| 5/25 | 91.1 | 5.87 | 8.1 | 9.4 | 35.8 | .65 | 36.7 |
| 5/31 | 92.9 | 5.46 | 7.9 | 10.9 | 34.5 | .83 | 34.1 |
| 6/6 | 92.0 | 5.96 | 7.9 | 12.0 | 36.5 | --- | 37.3 |
| 6/18 | 91.8 | 5.92 | --- | 12.3 | 32.5 | --- | 37.0 |
| 6/24 | 92.2 | 5.53 | 7.3 | 13.0 | 34.0 | --- | 34.6 |
| 7/1 | 92.6 | 5.58 | 8.6 | 14.0 | 32.0 | --- | 34.9 |
| 7/8 | 91.5 | 5.77 | 8.8 | 13.9 | 36.5 | --- | 36.1 |
| 7/15 | 92.2 | 5.25 | 10.0 | | 37.5 | --- | 32.8 |

[a] Averages of 4 channels except for 7/8 and 7/15 which are an average of 2.

[b] Nitrogen x 6.25.

TABLE 30. PARTIAL CHEMICAL ANALYSIS OF S. polyrhiza GROWN ON STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| Sample Date | % Moisture | % of dry matter[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | Fiber | Ash | TOC | Silica | Crude Protein[b] |
| 5/25 | --- | --- | --- | --- | --- | --- | --- |
| 5/31 | 94.3 | 5.38 | 9.5 | 13.1 | 34.3 | 1.25 | 33.6 |
| 6/6 | 94.2 | 6.55 | 8.6 | 12.9 | 34.3 | --- | 40.9 |
| 6/18 | 94.9 | 5.51 | --- | 13.0 | 33.8 | --- | 34.4 |
| 6/24 | 94.3 | 5.76 | 8.0 | 12.5 | 34.8 | --- | 36.0 |
| 7/1 | 94.7 | 5.95 | 9.4 | 12.6 | 34.8 | --- | 37.2 |
| 7/8 | 93.4 | 5.70 | 9.3 | 14.2 | 36.0 | --- | 35.6 |
| 7/15 | 93.0 | 5.44 | 9.6 | 12.9 | 32.5 | --- | 34.0 |

[a] Average of 4 channels except for 7/8 and 7/15 which are an average of 2.

[b] Nitrogen x 6.25.

species as well as several clones of a species so that some plants will be actively growing at all times.

With the reduction in growth in Test 2, the channels were reflooded with effluent from the stage 1 anaerobic lagoon to determine if the addition of nutrients would restimulate growth, and thereby improve treatment of the waste water. This study is shown in Test 4.

### Test 3, Mixing Treatment, Summer, 1976

Due to the low biomass of duckweed harvested during Test 2 and the reduced nutrient levels in the lagoon water, a test was set up to determine if mixing of the bottom sediments into the water column would revive duckweed growth. Analysis of the sludge TKN showed that 1300 to 1400 mg/l was present. Channels in test lagoon 2 were thoroughly mixed manually on alternate days and test lagoon 1 served as a non-mixed control. TABLES 31 and 32 show little change in water quality between the mixed and non-mixed channels. The TKN was the only value that showed a consistent increase in all channels as a result of mixing. However, the increase was modest, and by the end of two weeks the TKN values for the mixed and non-mixed channels had declined. Channels containing S. oligorhiza had similar TKN values under both conditions. S. polyrhiza and control channels were rapidly declining but the mixed channels showed higher TKN values. Values before mixing were not available for K and Ca. Potassium appeared a little higher in the non-mixed channel after the test started. Calcium was initially higher in the non-mixed channel but values were similar at the end.

The total suspended matter showed little change throughout the test. During the test, the sludge layer was thoroughly mixed throughout the water column, but rapidly settled. It is apparent that the sludge does not decompose rapidly as evidenced by little change in water quality values. The TKN in the sludge was about 30 X that in the water column, yet little change occurred due to mixing.

TABLES 33 and 34 show little change in biomass harvested as a result of mixing. In view of small nutrient change in the water, the lack of a biomass increase was not surprising. Chemical analysis of the duckweeds showed similar values as in Tests 1 and 2.

Because increased growth of the duckweeds failed to materialize, and nutrients did not increase in the water significantly, another test was set up to see if the addition of nutrients from the stage 1 anaerobic lagoon would stimulate growth. Test 4, below, describes the results.

### Test 4, Nutrient Addition and Growth, Summer, 1976

The objective of Test 4 was to determine if a nutrient increase would result in a growth improvement of duckweeds. It was thought that summer conditions should have resulted in higher growth than was achieved on the lagoons. Mixing of the bottom sediments failed to increase nutrients significantly and an improvement in growth was not evidenced. Thus effluent from the stage 1 lagoon, which received fresh water daily, was introduced

## TABLE 31. WATER QUALITY[a] OF TEST AND CONTROL CHANNELS UNDER MIXED[b] AND NON-MIXED CONDITIONS

| Date | TKN (mg/1) mixed | | | TKN (mg/1) non-mixed | | | K (mg/1) mixed | | | K(mg/1) non-mixed | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | S.o.[c] | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/1[d] | 38.7 | 39.0 | 38.9 | 30.1 | 28.8 | 34.5 | --- | --- | --- | --- | --- | --- |
| 7/2 | 43.0 | 43.5 | 43.1 | 33.8 | 33.5 | 35.5 | 77.6 | 73.7 | 79.6 | 88.1 | 83.8 | 96.2 |
| 7/8 | 33.6 | 37.1 | 37.4 | 24.0 | 26.0 | 28.5 | 81.0 | 73.3 | 84.4 | 80.1 | 84.4 | 93.4 |
| 7/15 | 23.4 | 29.5 | 27.8 | 23.3 | 17.1 | 20.7 | 69.8 | 63.6 | 74.3 | 75.4 | 72.6 | 81.0 |
| % reduction from 7/2 | 46 | 32 | 36 | 31 | 42 | 42 | 10 | 14 | 7 | 14 | 13 | 16 |

(Continued)

TABLE 31 (continued).

| Date | Ca(mg/1) mixed |  |  | Ca(mg/1) non-mixed |  |  | pH mixed |  |  | pH non-mixed |  |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/1 | --- | --- | --- | --- | --- | --- | 7.7 | 7.7 | 7.8 | 7.8 | 7.7 | 7.8 |
| 7/1 | 62.4 | 63.3 | 63.9 | 70.3 | 65.0 | 76.3 | 7.6 | 7.5 | 7.7 | 7.9 | 7.7 | 7.8 |
| 7/8 | 59.1 | 59.1 | 59.7 | 56.3 | 61.3 | 68.3 | 7.7 | 7.5 | 7.9 | 7.7 | 7.5 | 8.1 |
| 7/15 | 55.8 | 55.8 | 57.5 | 55.1 | 60.2 | 58.3 | 7.7 | 7.6 | 8.1 | 7.8 | 7.6 | 7.8 |
| % reduction from 7/2 | 11 | 12 | 10 | 22 | 7 | 24 | --- | --- | --- | --- | --- | --- |

(Continued)

TABLE 31 (continued).

| Date | TOC mixed |  |  | TOC non-mixed |  |  |
|---|---|---|---|---|---|---|
|  | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/2 | 85 | 83 | 83 | 82 | 78 | 85 |
| 7/8 | --- | --- | --- | --- | --- | --- |
| 7/15 | 78 | 88 | 76 | 83 | 84 | 85 |
| % reduction from 7/2 | 8 | --- | 8 | --- | --- | --- |

[a] Values are averages of 2 channels.

[b] Channels were mixed manually every other day.

[c] S.o. (S. oligorhiza), S.p. (S. polyrhiza), C (Control).

[d] Sample taken prior to mixing.

TABLE 32. CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF MIXED AND NON-MIXED CHANNELS[a]

| Date | *S. polyrhiza* | | | | | *S. oligorhiza* | | | | | Control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1976 | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM |
| | ----------------------------------------mg/1---------------------------------------- | | | | | | | | | | | | | | |
| Mixed | | | | | | | | | | | | | | | |
| 7/1 | 202 | 683 | 388 | 295 | 104 | 217 | 735 | 436 | 299 | 94 | 209 | 769 | 489 | 280 | 104 |
| 7/8 | 267 | 566 | 336 | 230 | 107 | 359 | 866 | 564 | 302 | 118 | 271 | 708 | 436 | 272 | 106 |
| 7/15 | 230 | 576 | 384 | 192 | 107 | 360 | 606 | 420 | 186 | 113 | 238 | 568 | 394 | 174 | 110 |
| Non-mixed | | | | | | | | | | | | | | | |
| 7/1 | 202 | 683 | 388 | 295 | 104 | 217 | 735 | 436 | 299 | 94 | 209 | 769 | 489 | 280 | 104 |
| 7/8 | 271 | 584 | 358 | 226 | 109 | 279 | 668 | 442 | 226 | 108 | 271 | 722 | 484 | 238 | 111 |
| 7/15 | 184 | 596 | 460 | 136 | 91 | 180 | 382 | 198 | 184 | 93 | 209 | 550 | 352 | 198 | 101 |

[a] Values on 7/1/76 are averages of 4 channels for each species and controls in test lagoons 1 and 2 prior to mixing. Values for 7/8 and 7/15 are averages of 2 channels each. Test lagoon 1 was non-mixed and test lagoon 2 was mixed.

TABLE 33. BIOMASS HARVEST OF *S. oligorhiza* AND *S. polyrhiza* GROWN ON MIXED AND NON-MIXED TEST CHANNELS, SUMMER, 1976

| | | Harvest date[a] | | | | Total/13 days | | Harvest/mo[c] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 7/8 | | 7/15 | | | | kg/ha | | lbs/acre | |
| | | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. |
| kg harvested/m$^2$[b] | | | | | | | | | | | |
| wet | mixed | .32 | .64 | .38 | .73 | .70 | 1.37 | --- | --- | --- | --- |
| | non-mixed | .35 | .50 | .41 | .79 | .76 | 1.29 | --- | --- | --- | --- |
| dry | (8.25%) mixed | .026 | .044 | .031 | .050 | .057 | .094 | 1315 | 2169 | 1174 | 1937 |
| | (8.90%) non-mixed | .031 | .035 | .037 | .055 | .068 | .090 | 1569 | 2077 | 1401 | 1855 |

[a] Values are averages of two test channels for each species under mixed and non-mixed conditions.

[b] Values show the average biomass removed (½) from a m$^2$.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

TABLE 34. CHEMICAL COMPOSITION OF *S. oligorhiza* AND *S. polyrhiza* GROWN ON MIXED AND NON-MIXED TEST CHANNELS, SUMMER, 1976[a]

| Sample Date | % moisture | | % of dry matter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | TKN | | Fiber | | Ash | | TOC | | Crude Protein[b] | |
| | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. | S.o. | S.p. |
| 7/8 | | | | | | | | | | | | |
| Mix | 91.3 | 93.4 | 5.77 | 5.70 | 8.8 | 9.3 | 14.0 | 14.2 | 36.5 | 36.0 | 36.1 | 35.6 |
| Non-mixed | 90.8 | 92.9 | 5.86 | 5.85 | 7.9 | 8.2 | 15.7 | 14.9 | 35.5 | 34.5 | 36.6 | 36.6 |
| 7/15 | | | | | | | | | | | | |
| Mix | 92.2 | 93.0 | 5.25 | 5.44 | 10.0 | 9.55 | 13.9 | 12.9 | 37.5 | 32.5 | 32.8 | 34.0 |
| Non-mixed | 91.4 | 93.0 | 5.55 | 5.72 | 8.6 | 9.35 | 16.7 | 13.5 | 35.0 | 36.0 | 34.7 | 35.8 |

[a] Average of 2 channels.

[b] Nitrogen x 6.25.

in an effort to increase the nutrients in the test channels. Test lagoon 1 was reflooded with stage 1 lagoon effluent on July 19 and 29. Test lagoon 2 was held static as a control.

TABLES 35, 36, 37, 38 and 39 show the chemical and bacteriological characteristics of the test and control channels during this test. It is evident that flooding the channels with waste effluent restulted in a nutrient increase. Data in these tables are primarily presented to show the extent of increase for comparison with duckweed growth. However, values for the static test channels provides some information on treatment at low water quality values. Under static conditions duckweed-covered channels showed a TKN reduction of 23% for _S. oligorhiza_, 52% for _S. polyrhiza_, and 24% in the control. Potassium, calcium, and pH changes were similar to previous tests. TKN reduction was less than 1 mg/l/day, which was less than previous reductions at higher nutrient levels. In TABLE 37 total, volatile, and fixed residues again are variable. These parameters have not appeared very useful in determining lagoon performance due to widely fluctuating values. The COD and total suspended matter, however, are less variable and may be useful in characterizing lagoon waste treatment along with TKN. Phosphorus, which has not been measured, may also prove useful and is evaluated in later tests.

TABLE 39 shows bacterial changes under the static condition at low nutrient levels. Although the values do fluctuate somewhat the trend to decline is evident, and thus are considered useful in evaluating lagoon performance.

TABLES 40 and 41 show that duckweed biomass between mixed and static channels were similar. Differences in biomass between species were insignificant. _S. oligorhiza_, which showed the lower harvest in Test 3 (mixing vs. non-mixing) increased considerably in Test 4. However, the controls which did not receive nutrients increased considerably as well. _S. polyrhiza_ had the higher biomass harvested in Test 3, but declined in Test 4 when fresh waste effluent was introduced into the test channels.

TABLES 42 and 43 indicate a different picture from the biomass change. Both species of duckweed had higher TKN values in the flooded channels than the static. Phosphorus appeared to increase also, while potassium, fiber, and ash declined. Thus it appears that with a nutrient increase in the lagoon water the plants accumulate higher levels of nutrients, but growth rates do not necessary change. The data in TABLES 34 and 21 indicate that less nitrogen is picked up when TKN in the waste water is below 25 to 30 mg/l. The ratio is dissolved nutrients may also affect plant nutrients and growth.

Management of lagoons for maximum uptake of nutrients by duckweeds must include consideration of nutrient quantity and possibly ratios of various nutrients. Further study is needed to relate plant growth and nutrient uptake with available nutrients before an effective management system can be recommended.

TABLE 35. WATER QUALITY[a] OF TEST AND CONTROL CHANNELS WITH AND WITHOUT THE ADDITION OF DAIRY WASTE EFFLUENT[b]

| | TKN (mg/1) flooded | | | TKN(mg/1) static | | | K(mg/1) flooded | | | K(mg/1) static | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | S.o.[c] | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/15[d] | 23.6 | 17.2 | 20.7 | 23.4 | 29.5 | 27.8 | 75.4 | 72.6 | 81 | 69.8 | 63.6 | 74.3 |
| 7/22 | 51.4 | 55.3 | 53.8 | 22.0 | 22.3 | 24.8 | 98.5 | 100.7 | 107.5 | 70.9 | 63.9 | 68.7 |
| 7/29 | 63.8 | 61.2 | 62.2 | 19.9 | 17.5 | 21.8 | 110.3 | 109.7 | 111.4 | 62.4 | 57.4 | 64.9 |
| 8/5 | 51.6 | 51.8 | 49.4 | 18.0 | 14.3 | 20.8 | 110.3 | 108.1 | 111.4 | 64.8 | 54.6 | 65.4 |

| | Ca(mg/1) flooded | | | Ca(mg/1) static | | | pH flooded | | | pH static | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/15 | 55.1 | 60.2 | 58.3 | 55.8 | 55.8 | 57.5 | 7.8 | 7.6 | 7.8 | 7.7 | 7.6 | 8.1 |
| 7/22 | 49.1 | 48.5 | 48.5 | 49.5 | 48.5 | 48.5 | 7.3 | 7.2 | 7.5 | 7.7 | 7.7 | 8.1 |
| 7/29 | 54.0 | 57.7 | 56.8 | 51.2 | 51.8 | 54.0 | 7.1 | 7.2 | 7.4 | 7.6 | 7.5 | 8.1 |
| 8/5 | 55.7 | 58.0 | 58.0 | 48.9 | 49.5 | 52.9 | 7.5 | 7.6 | 7.8 | 7.6 | 7.4 | 7.9 |

(Continued)

TABLE 35 (continued).

| Date | TOC(mg/1) flooded | | | TOC(mg/1) static | | |
|---|---|---|---|---|---|---|
| | S.o. | S.p. | C | S.o. | S.p. | C |
| 7/15 | --- | --- | --- | --- | --- | --- |
| 7/22 | 72 | 75 | 120 | 69 | 73 | 83 |
| 7/29 | 100 | 100 | 110 | 69 | 57 | 77 |
| 8/5 | 92 | 96 | 93 | 63 | 48 | 67 |

[a] Values are averages of two channels.

[b] Effluent was introduced on July 19 and 29.

[c] S.o. (S. oligorhiza); S.p. (S. polyrhiza); C (Control).

[d] Sample taken prior to addition of effluent.

TABLE 36. CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 1 RECEIVING DAIRY WASTE EFFLUENT

| Date | *S. polyrhiza* | | | | | *S. oligorhiza* | | | | | Control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1976 | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM |
| | mg/l | | | | | | | | | | | | | | |
| 7/15 | 230 | 576 | 384 | 192 | 107 | 360 | 606 | 420 | 186 | 113 | 238 | 568 | 394 | 174 | 110 |
| 7/22 | 322 | 948 | 581 | 367 | 146 | 385 | 916 | 710 | 206 | 136 | 322 | 860 | 652 | 208 | 144 |
| 7/29 | 353 | 998 | 580 | 418 | 90 | 288 | 852 | 614 | 238 | 80 | 346 | 1012 | 612 | 400 | 85 |
| 8/5 | 360 | 1048 | 606 | 442 | 60 | 338 | 1058 | 628 | 430 | 52 | 360 | 1116 | 698 | 418 | 68 |
| 8/13 | 187 | 798 | 444 | 354 | 51 | 166 | 729 | 428 | 301 | 48 | 187 | 782 | 441 | 341 | 53 |

TABLE 37. CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 2 HELD STATIC

| Date | *S. polyrhiza* | | | | | *S. oligorhiza* | | | | | Control | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1976 | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM |
| | mg/l | | | | | | | | | | | | | | |
| 7/15 | 184 | 596 | 460 | 136 | 91 | 180 | 382 | 198 | 184 | 93 | 209 | 550 | 352 | 198 | 101 |
| 7/22 | 188 | 760 | 666 | 94 | 78 | 200 | 808 | 702 | 106 | 128 | 220 | 764 | 560 | 204 | 88 |
| 7/29 | 187 | 752 | 556 | 196 | 22 | 180 | 732 | 388 | 344 | 27 | 169 | 794 | 400 | 394 | 39 |
| 8/5 | 266 | 810 | 492 | 318 | 32 | 216 | 806 | 440 | 366 | 60 | 230 | 958 | 500 | 458 | 84 |
| 8/13 | 108 | 770 | 404 | 366 | 31 | 94 | 604 | 392 | 212 | 26 | 94 | 594 | 388 | 206 | 32 |

TABLE 38. STANDARD PLATE COUNT (SPC), ANAEROBIC COUNT (AC), FECAL COLIFORM (FC), AND FECAL STREPTOCOCCI COUNTS (FSC) OF TEST LAGOON 1 RECEIVING DAIRY WASTE EFFLUENT

| | *S. polyrhiza* | | | | *S. oligorhiza* | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date 1976 | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml |
| | $\times 10^5$ | | | | | | | | | | | |
| 7/22 | 2.7 | 2.6 | 1.7 | 1.7 | 2.4 | 2.9 | 2.1 | 2.0 | 2.9 | 2.8 | 1.6 | 1.4 |
| 7/29 | 4.6 | 5.2 | 1.8 | 1.6 | 3.7 | 3.2 | 1.8 | 1.5 | 3.8 | 4.1 | 1.4 | 1.1 |
| 8/5 | 3.1 | 3.0 | 2.4 | 2.1 | 2.9 | 2.7 | 2.1 | 1.9 | 2.9 | 2.8 | 2.0 | 1.8 |
| 8/13 | 2.5 | 2.3 | 1.6 | 1.4 | 2.7 | 2.6 | 1.9 | 1.5 | 2.7 | 2.4 | 1.6 | 1.2 |

TABLE 39. STANDARD PLATE COUNT (SPC), ANAEROBIC COUNT (AC), FECAL COLIFORM (FC), AND FECAL STREPTOCOCCI COUNTS (FSC) OF TEST LAGOON 2 HELD STATIC

| | *S. polyrhiza* | | | | *S. oligorhiza* | | | | Control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date 1976 | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml | SPC/ ml | AC/ ml | FC/ 100 ml | FSC/ 100 ml |
| | x $10^5$ | | | | | | | | | | | |
| 7/22 | 1.7 | 1.5 | 1.7 | 1.4 | 1.3 | 1.1 | 2.3 | 1.9 | 1.1 | 1.0 | 1.8 | 1.6 |
| 7/29 | 1.7 | 1.6 | 1.6 | 1.3 | 1.3 | 1.3 | 1.8 | 1.6 | 1.4 | 1.2 | 1.9 | 1.4 |
| 8/5 | 1.5 | 1.3 | 1.2 | 1.1 | 1.7 | 1.2 | 2.7 | 2.4 | 1.3 | 1.2 | 2.2 | 1.9 |
| 8/13 | 0.7 | 0.6 | 1.1 | 1.1 | 0.8 | 0.9 | 1.9 | 1.4 | 0.8 | 0.7 | 1.5 | 1.3 |

TABLE 40. *S. oligorhiza* BIOMASS HARVESTED FROM FLOODED AND STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| | | Harvest date[a] | | Total/16 days | Harvest/mo[c] | |
|---|---|---|---|---|---|---|
| | | 7/30 | 8/4 | | kg/ha | lbs/acre |
| kg harvested/$m^2$[b] | | | | | | |
| wet | flooded | .66 | .65 | 1.31 | --- | --- |
| | static | .50 | .61 | 1.11 | --- | --- |
| dry (7.95%) | flooded | .053 | .052 | .105 | 1969 | 1758 |
| (8.78%) | static | .044 | .054 | .098 | 1838 | 1641 |

[a] Values as averages of 2 test channels for each species under flooded and static conditions.

[b] Values show the average biomass removed (½) from a $m^2$.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

TABLE 41. S. polyrhiza BIOMASS HARVESTED FROM FLOODED AND STATIC TEST CHANNELS RECEIVING DAIRY WASTE, SUMMER, 1976

| | | Harvest date[a] | | Total/16 days | Harvest/mo[c] | |
|---|---|---|---|---|---|---|
| | | 7/30 | 8/4 | | kg/ha | lbs/acre |
| kg harvested/m$^2$[b] | | | | | | |
| wet | flooded | .67 | .63 | 1.30 | --- | --- |
| | static | .64 | .71 | 1.35 | --- | --- |
| dry (6.36%) | flooded | .043 | .040 | .083 | 1556 | 1390 |
| (6.89%) | static | .044 | .049 | .049 | 1744 | 1557 |

[a] Values as averages of 2 test channels for each species under flooded and static conditions.

[b] Values show the average biomass removed (½) from a m$^2$.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

TABLE 42. CHEMICAL COMPOSITION OF *S. oligorhiza* GROWN ON FLOODED AND STATIC TEST CHANNELS, SUMMER, 1976[a]

| Sample Date | % moisture | % of dry matter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | TKN | P | K | Fiber | Ash | TOC | Crude Protein[b] |
| 7/30 | | | | | | | | |
| Flooded | 92.05 | 6.19 | 1.49 | 1.75 | 7.30 | 7.5 | 33.5 | 38.9 |
| Static | 91.17 | 4.84 | 1.46 | 2.00 | 7.35 | 8.9 | 32.5 | 30.1 |
| 8/4 | | | | | | | | |
| Flooded | 92.06 | 5.78 | 1.45 | 1.85 | 5.90 | 7.9 | 35.0 | 36.1 |
| Static | 91.28 | 5.19 | 1.49 | 2.34 | 8.25 | 9.4 | 31.0 | 32.4 |

[a] Average of 2 channels.

[b] Nitrogen x 6.25.

TABLE 43. CHEMICAL COMPOSITION OF *S. polyrhiza* GROWN ON FLOODED AND STATIC TEST CHANNELS, SUMMER, 1976[a]

| Sample Date | % moisture | % of dry matter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | TKN | P | K | Fiber | Ash | TOC | Crude Protein[b] |
| 7/30 | | | | | | | | |
| Flooded | 93.49 | 6.30 | 1.44 | 1.75 | 7.70 | 8.25 | 34.0 | 39.4 |
| Static | 93.09 | 5.02 | 1.22 | 2.70 | 9.00 | 8.70 | 34.0 | 31.4 |
| 8/4 | | | | | | | | |
| Flooded | 93.79 | 6.08 | 1.35 | 1.83 | 7.45 | 8.90 | 32.0 | 38.0 |
| Static | 93.13 | 5.49 | 1.23 | 2.78 | 8.50 | 9.10 | 33.0 | 34.3 |

[a] Average of 2 channels.

[b] Nitrogen x 6.25.

### Test 5, Duckweed Growth and Nutrient Uptake, Late Summer-Early Fall, 1976

As a follow-up to test 4 to determine if the addition of fresh effluent would stimulate duckweed growth and nutrient uptake, test lagoon 2 was stocked with two duckweed cultures on three channels each. *S. polyrhiza* had a mixed culture of *S. polyrhiza* and *S. oliforhiza* were tested. Data was useful for comparison with spring data (Test 1) as air temperatures were similar. Test channels were flooded once weekly.

TABLES 44 and 45 show chemical analysis of test channel water throughout the study. Due to the addition of effluent from the stage 1 lagoon water quality values tended to fluctuate, depending on changes with the stage 1 lagoon.

TABLE 46 shows growth of *S. polyrhiza* and the mixed culture for only a 7 day period. Two harvests were taken, on September 1 and 8. However, the second harvest was not weighed, as they were accidentally removed for feeding before weights were taken. Growth during the second week appeared similar to the first. Samples for chemical analysis were taken however.

Biomass harvested equaled or exceeded previous harvest data and strengthens the indication that continuous or periodic flooding improves growth.

TABLES 47 and 48 show chemical content of the duckweed cultures. Boron was included in the analysis as it is an ingredient in detergents used in the milking operation. It was picked up by the duckweeds as evidenced in the tables, but in low concentration, and should pose no problems to animal utilizing duckweed as part of a standard diet.

Phosphorus, TKN, K and fats increased over the test, while calcium declined, and fiber, ash, and TOC fluctuated slightly. The data again shows that periodic flooding with nutrient-rich effluent results in an increase in TKN and protein.

### Test 6, Waste Treatment and Duckweed Growth Under Aeration, Fall, 1976

In September, 1976, unseasonably cool weather developed. Duckweed growth declined significantly, but the plants remained on the surface. In order to obtain information on waste treatment in lagoons and plant growth during winter-time conditions a test was set up November 3 and carried to December 4. Three channels were used for each: *S. oligorhiza*, *S. polyrhiza*, a mixture of the two, and controls. The system was aerated on alternate days to obtain information on treatment characteristics under aerobic low temperature conditions with and without duckweed.

TABLES 49 through 53 show water quality conditions during this study. Several days of below freezing temperatures occurred, but not sufficiently low to freeze over the lagoons. The channels were slow in responding to aeration, as most channels showed little oxygen increase until near the end of the test. Some of the channels were well oxygenated for 15 to 20 days but the conversion of ammonium to nitrites and nitrates was not clearly

TABLE 44. WATER QUALITY[a] OF TEST LAGOON 2 RECEIVING DAIRY WASTE EFFLUENT INTERMITTENTLY[b] AND SUPPORTING A STAND OF S. *polyrhiza* AND A MIXED STAND OF S. *polyrhiza* AND S. *oligorhiza*, LATE SUMMER, 1976

| Date[c] | TKN (mg/l) |  | K (mg/l) |  | Ca (mg/l) |  | pH |  |
|---|---|---|---|---|---|---|---|---|
|  | S.p. | mixed | S.p. | mixed | S.p. | mixed | S.p. | mixed |
| 8/26 | 31.0 | 33.7 | 75.0 | 78.0 | 56.3 | 54.4 | 7.2 | 7.2 |
| 9/1 | 28.8 | 29.4 | 63.0 | 67.5 | 45.0 | 47.3 | --- | --- |
| 9/8 | 41.2 | 35.0 | 69.8 | 66.4 | 47.3 | 47.3 | 7.1 | 7.2 |

[a] Values are averages of 3 channels.

[b] Effluent was added August 26, 31, and September 7.

[c] Sample taken after effluent added.

TABLE 45. CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF TEST LAGOON 2, FLOODED INTERMITTENTLY[a] WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976

| | *S. polyrhiza* | | | | | Mixed Culture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Date[c] | [b]COD | TR | VR | FR | TSM | COD | TR | VR | FR | TSM |
| | mg/1 | | | | | | | | | |
| 8/26 | 97 | 406 | 301 | 105 | 63 | 117 | 419 | 311 | 108 | 70 |
| 9/1 | 186 | 431 | 257 | 174 | 40 | 198 | 551 | 428 | 123 | 50 |
| 9/8 | 232 | 760 | 560 | 200 | 59 | 199 | 753 | 511 | 242 | 64 |

[a] Effluent added August 26, 31, and September 7.

[b] Values represent 3 channels combined.

[c] Sample taken after effluent added.

TABLE 46. BIOMASS OF *S. polyrhiza* AND A MIXED CULTURE OF *S. polyrhiza* AND *S. oligorhiza* HARVESTED[a] FROM TEST LAGOON 2 FLOODED INTERMITTENTLY WITH DAIRY WASTE EFFLUENT[b], LATE SUMMER, 1976

| | Harvest date 9/1 | | Total harvested/ 7 days | | Harvest/mo/ | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | kg/ha | | lbs/acre | |
| | S.p. | mixed | S.p. | mixed | S.p. | mixed | S.p. | mixed |
| kg harvested/$m^2$[c] | | | | | | | | |
| wet | .69 | .69 | .69 | .69 | --- | --- | --- | --- |
| dry (7.3%) | .05 | --- | .05 | --- | 2156 | --- | 1925 | --- |
| (7.3%) | --- | .05 | --- | .05 | --- | 2156 | --- | 1925 |

[a] Average of 3 channels.

[b] Effluent added August 26, 31, and September 7.

[c] Values show the average biomass removed (½) from a $m^2$.

TABLE 47. CHEMICAL ANALYSIS OF S. polyrhiza GROWN ON TEST CHANNELS PERIODICALLY FLOODED WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976[a]

| Sample Date | % moisture | % of dry matter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TKN | P | K | Ca | Fiber | Ash | Fat | TOC | B[b] | Crude Protein[c] |
| 8/25[d] | 92.0 | 4.87 | 1.10 | 1.90 | 2.38 | 8.6 | 20.7 | 4.2 | 34.3 | .022 | 30.4 |
| 9/1 | 93.4 | 5.64 | 1.21 | 1.98 | 2.42 | 8.9 | 17.2 | 7.8 | 33.7 | .029 | 35.3 |
| 9/8 | 92.7 | 5.84 | 1.37 | 2.08 | 2.17 | 8.7 | 17.2 | 8.6 | --- | .024 | 36.5 |

[a] Average of 3 channels.

[b] Boron.

[c] TKN x 6.25.

[d] This sample taken prior to adding effluent (August 26, 31 and September 7).

TABLE 48. CHEMICAL ANALYSIS OF A MIXED STAND OF S. oligorhiza AND S. polyrhiza GROWN ON TEST CHANNELS PERIODICALLY FLOODED WITH DAIRY WASTE EFFLUENT, LATE SUMMER, 1976[a]

| Sample Date | % moisture | % of dry matter | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TKN | P | K | Ca | Fiber | Ash | Fat | TOC | B[b] | Crude Protein[c] |
| 8/25[d] | 92.4 | 5.44 | 1.28 | 2.03 | 2.18 | 8.0 | 18.0 | 6.4 | 32.2 | .028 | 34.0 |
| 9/1 | 93.0 | 5.64 | 1.38 | 2.43 | 1.69 | 9.1 | 15.9 | 9.2 | 32.7 | .034 | 35.3 |
| 9/8 | 93.4 | 6.07 | 1.68 | 2.50 | 1.67 | 8.8 | 17.8 | 8.3 | --- | .034 | 38.0 |

[a] Average of 3 channels.

[b] Boron.

[c] TKN x 6.25.

[d] This sample taken prior to adding effluent (August 26, 31 and September 7).

TABLE 49. WATER QUALITY[a] OF AERATED, STATIC LAGOON TEST CHANNELS SUPPORTING STANDS OF *S. polyrhiza*, LATE FALL, 1976

| Sample Date | mg/1 | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | pH | Surface Temp °C |
| 11/3 | 47.9 | <.25 | <.1 | 26.0 | 31.9 | 0 | 7.4 | 14 |
| 11/4 | --- | " | " | 30.4 | 33.6 | 0 | --- | " |
| 11/6 | --- | " | " | 29.0 | --- | 0.3 | --- | " |
| 11/8 | --- | " | " | 32.6 | --- | 0 | --- | --- |
| 11/10 | --- | " | " | 29.6 | 32.3 | 0.1 | --- | 19 |
| 11/15 | --- | " | .3 | 29.6 | --- | 5.5 | --- | 8 |
| 11/20 | 34.6 | " | 1.1 | 29.3 | 31.8 | 1.6 | 7.9 | 9 |
| 11/30 | 29.1 | .25 | 2.0 | 29.4 | 26.2 | 7.9 | 7.9 | --- |
| 12/4 | 26.7 | .82 | 1.0 | 24.0 | 24.1 | 7.4 | --- | --- |

[a] Average of 2 or 3 channels.

TABLE 50. WATER QUALITY[a] OF AERATED STATIC LAGOON TEST CHANNELS SUPPORTING A STAND OF *S. oligorhiza*, LATE FALL, 1976

| Sample Date | mg/l |  |  |  |  |  | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
|  | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ |  |  |
| 11/3 | 54.9 | <.25 | <.1 | 32.5 | 34.5 | 0 | 7.3 | 14 |
| 11/4 | --- | " | " | 31.2 | 34.8 | 0 | --- | " |
| 11/6 | --- | " | " | 33.6 | --- | 0 | --- | " |
| 11/8 | --- | " | " | --- | 34.0 | 2.3 | --- | --- |
| 11/10 | --- | " | " | 30.4 | 34.3 | 0.13 | --- | 19 |
| 11/15 | --- | " | " | --- | 32.6 | 0.9 | --- | 8 |
| 11/20 | 39.6 | " | .1 | 29.9 | 35.3 | 0.9 | 7.8 | 9 |
| 11/30 | 32.5 | " | .15 | 30.4 | 28.6 | 4.4 | 7.8 | --- |
| 12/4 | 32.6 | " | .1 | --- | 24.7 | 3.4 | --- | --- |

---

[a] Average of 2 or 3 channels.

TABLE 51. WATER QUALITY[a] OF AERATED STATIC LAGOON TEST CHANNELS SUPPORTING A MIXED STAND OF *S. oligorhiza* AND *S. polyrhiza*, LATE FALL, 1976

| Sample Date | mg/1 | | | | | | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | | |
| 11/3 | 51.1 | <.25 | <.1 | 27.3 | 34.3 | 0 | 7.4 | 14 |
| 11/4 | --- | " | " | 30.5 | 35.0 | 0 | --- | " |
| 11/6 | --- | " | " | 29.5 | --- | 0 | --- | " |
| 11/8 | --- | " | " | --- | 34.4 | .1 | --- | --- |
| 11/10 | --- | " | " | 28.5 | 34.0 | 1.2 | --- | 19 |
| 11/15 | --- | " | " | --- | 31.6 | .3 | --- | 8 |
| 11/20 | 37.3 | " | .2 | 29.0 | 31.4 | 1.3 | --- | 9 |
| 11/30 | 31.5 | " | .3 | 29.1 | 24.6 | 3.8 | 7.9 | --- |
| 12/4 | 27.9 | .35 | .75 | --- | --- | 7.3 | --- | --- |

---

[a] Average of 2 or 3 channels.

TABLE 52. WATER QUALITY[a] OF AERATED STATIC LAGOON CONTROL CHANNELS, LATE FALL, 1976

| Sample Date | mg/1 | | | | | | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | | |
| 11/3 | 51.3 | <.25 | <.1 | 31.1 | 32.3 | 0 | 7.4 | 14 |
| 11/4 | --- | " | " | 29.2 | 34.4 | 0 | --- | " |
| 11/6 | --- | " | " | 30.6 | --- | 0 | --- | " |
| 11/8 | --- | " | " | --- | 34.5 | .15 | --- | --- |
| 11/10 | --- | " | " | 28.2 | 33.1 | 0 | --- | 19 |
| 11/15 | --- | " | " | --- | 33.4 | 2.9 | --- | 8 |
| 11/20 | 38.9 | .04 | .15 | 28.2 | 33.2 | 1.9 | 7.9 | 9 |
| 11/30 | 33.3 | .3 | .35 | 28.4 | 27.2 | 8.1 | 7.9 | --- |
| 12/4 | 30.8 | .41 | .15 | --- | 26.1 | 6.8 | --- | --- |

[a] Average of 2 or 3 channels.

TABLE 53. BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE[a] SAMPLES OF TEST LAGOONS 1 AND 2

| Date | *S. polyrhiza* | | | *S. oligorhiza* | | | Mixed Culture | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1976 | BOD | COD | TSM | BOD | COD | TSM | BOD | COD | TSM | BOD | COD | TSM |
| | mg/1 | | | | | | | | | | | |
| 11/4 | 68 | 267 | 198 | 69 | 273 | 194 | 63 | 319 | 201 | 59 | 261 | 173 |
| 11/24 | 48 | 206 | 340 | 28 | 170 | 186 | 18 | 174 | 184 | 48 | 206 | 226 |
| 11/30 | 32 | 182 | 162 | 29 | 186 | 155 | 30 | 192 | 156 | 36 | 193 | 181 |

| Date | *S. polyrhiza* | | | *S. oligorhiza* | | | Mixed Culture | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1976 | TR | VR | FR | TR | VR | FR | TR | VR | FR | TR | VR | FR |
| 11/4 | 1027 | 566 | 461 | 1022 | 556 | 466 | 1046 | 491 | 555 | 1006 | 563 | 443 |
| 11/24 | 988 | 514 | 474 | 944 | 520 | 424 | 798 | 458 | 340 | 954 | 486 | 468 |
| 11/30 | 704 | 273 | 431 | 721 | 323 | 398 | 819 | 472 | 347 | 816 | 470 | 346 |

[a] Average of 3 channels.

evident. Control and test channels had similar TKN, BOD, COD, TR, VR, FR, and TSM values. TKN decline in the test channels averaged 43% while the controls showed a decline of 41%. Nitrates and Nitrites showed small increases throughout the test period indicating the conversion of ammonium was slow. Phosphorus values indicate that the duckweed-covered channels may have an effect on phsophorus decline, as these channels showed an average decline of 27% while the controls averaged 19% decline. However, due to the lack of growth of the plants the decline is probably not related to phosphorus uptake. Considering the low temperatures and lower metabolic activity, the decline in all channels was rather sharp.

TABLE 54 shows the duckweed biomass harvested. Due to freezing temperatures biomass increase did not occur. Thus harvest indicates only what was removed and should not be taken as an indication of biomass replacement.

TABLE 55 shows the nutrient content in the plants. In spite of the low growth rate nutrient values remained similar to previous values.

### Test 7, Winter Growth of Duckweeds, Covered and Non-covered, Winter, 1976-1977

During the fall of 1976 *S. oligorhiza* growth declined greatly. From December 10, 1976 through January 7, 1977 a preliminary study was set up to compare growth of *S. oligorhiza* under covered channels as growth had declined in Test 6 due to freezing temperatures. Three channels of *S. oligorhiza* were covered with .006 mm clear polyethylene plastic and four channels (not covered) served as non-stocked controls to compare treatment effects. The clone of *L. gibba* which had been stocked during the summer showed rapid growth during the fall. Three channels of *L. gibba*, non-covered, were added to obtain information on growth of this species under winter conditions. All channels were aerated to insure nutrient mixing.

Tests 3, 4, 5, and 6, which involved circulation of nutrients in the lagoons, failed to stimulate significant growth of duckweeds, except possibly in channels where the C:N ratio was 2:1 less. Aeration in the fall, when cooler temperatures prevailed did not produce a significant growth increase. Temperature, rather than low nutrients, seemed to be in the factor most likely affecting growth. By covering the test channels in Test 7, we hoped to determine if elevation of temperature would result in a growth increase, and be reflected in waste treatment.

TABLES 56, 57, 58 and 59 show the water quality of the test and control channels for *S. oligorhiza* covered channels was 1 to 2°C higher than controls. Air temperatures under the cover were variable but exceeded 21°C on several occasions and did not drop below freezing as did the ambient temperature on several occasions during the study. Reduction in TKN, $NH_4^+$, and P was greater in the test channels for both species.

TABLE 54. BIOMASS OF S. oligorhiza, S. polyrhiza, AND A MIXED (M) CULTURE OF THE TWO HARVESTED FROM AERATED[a] STATIC TEST LAGOONS, LATE FALL, 1976

| | Harvest date[b] | | | | | | Total harvest/ 31 days | | | Harvest/mo[c] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 11/25 | | | 12/4 | | | | | | kg/ha | | | lbs/acre | | |
| | S.o. | S.p. | M | S.o. | S.p. | M | S.o. | S.p. | M | S.o. | S.p. | M | S.o. | S.p. | M |
| kg harvested/m$^2$[d] | | | | | | | | | | | | | | | |
| wet | .29 | .38 | .51 | .19 | .17 | .27 | .48 | .55 | .78 | --- | --- | --- | --- | --- | --- |
| dry (8%) | .023 | | | .015 | | | .038 | | | 368 | | | 328 | | |
| (6%) | | .023 | | | .014 | | | .037 | | | 358 | | | 320 | |
| (7%) | | | .036 | | | .019 | | | .055 | | | 532 | | | 475 |

[a] Channels aerated every other day.

[b] Average of 3 channels, stocked 11/3/76.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

[d] Values show the average biomass removed (½) from a m$^2$.

TABLE 55. CHEMICAL ANALYSIS OF *S. oligorhiza* AND *S. polyrhiza* GROWN ON AERATED STATIC TEST CHANNELS RECEIVING DAIRY WASTE, LATE FALL, 1976

| Sample Date | % moisture | % of dry matter[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | TKN | Fiber | Ash | P | K | Ca | Fats | Crude Protein[b] |
| 12/4 | | | | | | | | | |
| *S. oligorhiza* | 92.0 | 5.97 | 8.8 | 10.8 | 1.32 | 1.77 | .12 | 4.0 | 37.3 |
| *S. polyrhiza* | 94.0 | 5.62 | 6.7 | 18.2 | 1.50 | 1.48 | .15 | 3.5 | 35.1 |
| mixed | 93.0 | 5.51 | 6.8 | 18.3 | 1.42 | 1.68 | .11 | 3.8 | 34.4 |

[a] Average of 3 channels.

[b] Nitrogen x 6.25.

TABLE 56. WATER QUALITY OF AERATED, STATIC LAGOON TEST CHANNELS SUPPORTING STANDS OF S. *oligorhiza*[a], WINTER, 1976-77

| Sample Date | mg/1 TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
| 12/10 | 59.8 | <.25 | .1 | 41.7 | 28.8 | --- | 7.5 | --- |
| 12/16 | --- | " | --- | 41.3 | 27.6 | --- | 7.6 | --- |
| 12/23 | 44.0 | " | .7 | 35.4 | 26.9 | 1.6 | 7.8 | 10.3 |
| 12/29 | 37.0 | " | 2.0 | 28.9 | 25.8 | .93 | 7.8 | 10.5 |
| % change | -38 | --- | +1900 | -31 | -10 | | | |

[a] Covered with clear plastic, average of 3 channels.

TABLE 57. WATER QUALITY OF AERATED, STATIC LAGOON TEST CHANNELS, CONTROLS[a], WINTER, 1976-77

| Sample Date | mg/1 | | | | | | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | | |
| 12/10 | 54.9 | <.25 | .13 | 38.6 | 28.8 | --- | 7.6 | --- |
| 12/16 | --- | " | --- | 38.3 | 28.6 | --- | 7.8 | --- |
| 12/23 | 43.6 | " | .23 | 35.6 | 28.7 | 2.2 | 7.9 | 8.0 |
| 12/29 | 40.6 | " | .33 | 31.5 | 28.3 | 2.5 | 8.0 | 9.6 |
| % change | -26 | --- | +154 | -18 | -2 | | | |

[a] Non-covered, average of 3 channels.

TABLE 58. WATER QUALITY OF AERATED, STATIC TEST CHANNELS SUPPORTING STANDS OF *L. gibba*[a], WINTER, 1976-77

| Sample Date | mg/1 | | | | | | pH | Surface Temp °C |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | | |
| 12/10 | 48.7 | <.25 | <.1 | 37.5 | 21.8 | --- | 7.5 | --- |
| 12/16 | --- | " | --- | 35.8 | 22.3 | --- | 7.6 | --- |
| 12/23 | 30.9 | " | <.1 | 21.0 | 22.3 | .8 | 7.8 | 7.8 |
| 12/29 | 23.8 | " | .3 | 17.1 | 20.5 | .7 | 7.8 | 7.8 |
| % change | -51 | --- | +200 | -54 | -6 | | | |

[a] Non-covered, average of 3 channels; from Dr. William Hillman, Brookhaven National Laboratory, Upton, N.Y., clone designation (G-3).

TABLE 59. WATER QUALITY OF AERATED, STATIC TEST CHANNELS, CONTROLS[a], WINTER, 1976-77

| Sample Date | mg/1 | | | | | | | Surface Temp $^oC$ |
|---|---|---|---|---|---|---|---|---|
| | TKN | $NO_3$ | $NO_2$ | $NH_4$ | P-ortho as $PO_4$ | $O_2$ | pH | |
| 12/10 | 39.8 | <.25 | <.1 | 28.0 | 24.3 | --- | 7.5 | --- |
| 12/16 | --- | " | --- | 28.5 | 25.0 | --- | 7.5 | --- |
| 12/23 | 40.8 | " | <.1 | 40.0 | 28.5 | .2 | 7.5 | 7.5 |
| 12/29 | 43.3 | " | .3 | 29.7 | 26.3 | .5 | 7.8 | 7.8 |
| % change | +9 | --- | +200 | +6 | +8 | | | |

[a] Non-covered, average of 3 channels.

The higher temperature under cover was associated with an increase in growth (TABLE 60) for *S. oligorhiza* over previous data (Test 6). The greater inprovement in water quality under the plastic-covered channels appeared unusual if temperature related, because of the slight differences between test and control channels. *L. gibba* channels showed a performance similar to *S. oligorhiza* with greater TKN, $NH_4^+$, and P reductions than controls. *L. gibba*, exposed to lower temperatures than *S. oligorhiza*, had as rapid growth, indicating the plant is more cold tolerant.

TKN reduction for the *S. oligorhiza* channels average 1.2 mg/l/day for 19 days, and *L. gibba* channels averaged 1.3 mg/l/day. These values equal or exceed the rate of decline in previous tests, and exceed the controls which respectively increased in one case (TABLE 59) and declined .75 mg/l/day in the second (TABLE 57).

Harvest data (TABLE 60) show that the addition of minimal amount of heat can improve growth of *S. oligorhiza* during winter, and the growth may be due to increased air temperature under the cover rather than in water temperature. Growth of *L. gibba* without being covered equaled that of *S. oligorhiza*, indicating this plant is cold tolerant, and can be utilized during winter months if maintained in lagoons free of ice cover. Estimated harvest for both species/hectare was sufficient to warrant further study on winter growth, particularly since the winter of 1976-77 was one of the most severe on record.

TABLE 61 shows that the nutrient content of both plants was similar to previous values. The ash values were in part due to silica. It is not known if the silica was taken up by the plants or due to inadequate washing before analysis. *L. gibba* appears to concentrate potassium, which could limit its use as a cattle feed. The increased productivity over Test 6 shows the potential for 12 month production by selecting appropriate clones, or protection from low temperatures.

### Test 8, Duckweed Growth and Water Quality in Covered and Non-covered Channels, Winter, 1977

Results of Test 7 showed that growth of *L. gibba* exposed to normal winter air temperatures equaled *S. oligorhiza* which was covered with clear polyethelene. During Test 7 several freezes caused the non-covered ponds to ice over. Test 8 was set up to determine if *L. gibba* was offered protection from freezing temperatures, would an increase in growth occur. Three test channels with the plant were covered as in Test 7 and three remained open. All six channels were stocked with 9.09 kg of the plant on February 8, 1977 and continued until March 21. Three non-covered channels with a mixture of *L. gibba* and *S. oligorhiza* were included to provide further information on the growth of mixed cultures. Three non-stocked, open channels served as controls. Aeration was applied to all channels, but failure of the air pump after the first harvest date terminated aerated-nonaerated comparisons except for the first harvest. All data collected up to the first harvest period are averages of the two non-aerated channels; thereafter results are the average of three channels, all non-aerated.

TABLE 60. BIOMASS OF *S. oligorhiza*[a] AND *L. gibba*[b] HARVESTED FROM AERATED, STATIC TEST LAGOONS, WINTER, 1976-77

| | Stocked/$m^2$ 12/10/76 | | Total /harvest 28 days 1/7/77 | | Harvest/mo[c] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | kg/ha | | lbs/acre | |
| | S.o. | L.g. | S.o. | L.g. | S.o. | L.g. | S.o. | L.g. |
| kg /$m^2$[d] | | | | | | | | |
| wet | .35 | .33 | .89 | .93 | --- | --- | --- | --- |
| dry (8%) | | | .071 | | 760.7 | | 679.3 | |
| (6%) | | | | .056 | | 611.0 | | 545.6 |

[a] Covered with clear plastic.

[b] Non-covered, see Table 58 for source of plant.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

[d] Average of 3 channels; values show the biomass stocked and removed (½) from a $m^2$.

TABLE 61. CHEMICAL ANALYSIS OF *S. oligorhiza*[a] AND *L. gibba*[b] GROWN ON AERATED STATIC TEST CHANNELS RECEIVING DAIRY WASTE, WINTER, 1976-77

| | % of dry matter | | | | |
|---|---|---|---|---|---|
| TKN[c] | | Ash | | Moisture | |
| S.o. | 6.04 | S.o. | 15.7 | S.o. | 92 |
| L.g. | 6.16 | L.g. | 16.4 | L.g. | 94 |
| P | | Fat | | | |
| S.o. | 1.45 | S.o. | 3.8 | | |
| L.g. | 1.55 | L.g. | 3.0 | | |
| K | | Silica | | | |
| S.o. | 2.86 | S.o. | 5.4 | | |
| L.g. | 4.19 | L.g. | 4.6 | | |
| Ca | | Crude protein[d] | | | |
| S.o. | 1.3 | S.o. | 37.8 | | |
| L.g. | 1.0 | L.g. | 38.5 | | |
| Fiber | | | | | |
| S.o. | 7.3 | | | | |
| L.g. | 9.4 | | | | |

[a] Covered with clear plastic.

[b] Non-covered.

[c] Based on plants collected (1/7/77), the last day of testing. Test began December 10, 1976. Values are averages of 3 channels.

[d] Nitrogen x 6.25.

Although not shown in the tables, plant growth between aerated and non-aerated channels at the first harvest date agreed with previous tests that aeration offered no advantage and may reduce plant growth.

Yield data are presented in TABLE 62. Both L. gibba treatments yielded approximately 1400 kg/ha/mo. Thus covering of the plant was of little value during this experiment. The mixed culture did not show as high an estimated yield, but analysis of variance confirmed that the difference was not significant ($P < .05$).

The fact that no significant difference of yield was observed between the mixed culture and monoculture treatments is of potential importance to management of lagoon systems using aquatic plants as part of the treatment process. By utilizing a mixture of clones selected for optimal growth (seasonally) and nutrient content, one species and/or clone would become dominant or less active for any given set of environmental conditions.

Chemical composition of the plants for the test period are shown in TABLE 63. It appears that there are differences in values as compared to previous experiments. Ash content is noticeably higher and is most likely due to the samples having not been thoroughly washed.

Water content in L. gibba increased somewhat over previous tests. We think this may be accounted for by the less active growth, and evidence of slight deterioration of some individual plants. This species contains large air cells on the lower surface. There was evidence of cell wall deterioration and the cells were found to contain water.

Duckweeds, during winter, frequently become dormant. In so doing they show an increase in starch and each frond separates from the present plant and sinks to the bottom. These dormant fronds (called turions) sink due to the accumulation of starch. The reduction in carbohydrate, as estimated from the nitrogen free extract (NFE), through study is puzzling as just the opposite would be expected. More curious is the increase in lipids as this would tend to make the plant more bouyant.

Reduction in TKN through the test, based on previous work, can be explained by the low nutrient content in the lagoon, as earlier tests indicated a drop in plant nitrogen when lagoon TKN was less than 20-30 mg/l.

TABLES 64 and 65 show the water quality for the non-covered mixed duckweed and control channels. Test channels performed significantly better than controls as in other tests. Comparisons between the aerated and static channels after February 25 cannot be made due to the breakdown of the aeration system. Little differences were noted on February 25 after twelve days of aeration, except for TKN, in which the static test and control channels showed greater TKN reductions. These data, again point out the questionable value of aeration as a treatment technique. As a management technique for increasing duckweed growth, aeration alone doesn't appear to offer any advantages.

TABLE 62. YIELD[a] OF L. gibba, COVERED AND NON-COVERED AND A MIXED CULTURE (M) OF L. gibba AND S. oligorhiza HARVESTED FROM STATIC TEST CHANNELS RECEIVING DAIRY WASTE, WINTER, 1977

| | Harvest date[b] | | | | | | | | | | | | Total/41 days | | | Harvest/mo[d] kg/ha[e] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2/25/77 | | | 3/7/77 | | | 3/16/77 | | | 3/21/77 | | | | | | | | |
| | L.g. | L.g.3[c] | M | L.g. | L.g.3 | M | L.g. | L.g.3 | M | L.g. | L.g.3 | M | L.g. | L.g.3 | M | L.g. | L.g.3 | M |
| wet | 0.673 | 0.573 | 0.391 | 0.624 | 0.833 | 0.573 | 1.182 | 1.124 | 1.085 | 0.824 | 0.745 | 0.776 | 3.303 | 3.275 | 2.825 | --- | --- | --- |
| dry (5.83%) | 0.039 | 0.033 | 0.023 | 0.036 | 0.049 | 0.033 | 0.069 | 0.066 | 0.063 | 0.048 | 0.043 | 0.045 | 0.192 | 0.191 | 0.164 | 1405 | 1398 | 1200 |

[a] Yield data reflects biomass removed per $m^2$ from one-half (12.5 $m^2$) of each test channel, one half was left for regrowth.

[b] Means of 3 test channels except for 2/25/77 which is the mean of two channels.

[c] Channels covered with polyethelene plastic.

[d] Harvest/mo based on 30 day month and represents Kg harvested from one-half of a hectare.

[e] Multiply by 0.893 to obtain lbs/ac/mo.

TABLE 63. PARTIAL CHEMICAL COMPOSITION OF *L. gibba* (G3) AND A MIXED CULTURE (M) OF *L. gibba* AND *S. oligorhiza* GROWN ON STATIC TEST CHANNEL RECEIVING DAIRY WASTE, WINTER, 1977

| Sample date[a] | % moisture | % of dry matter | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TKN | P | K | Ca | Fiber | Ash | Fats | NFE[b] | Crude protein[c] |
| Stocked 2/8/77 | | | | | | | | | | |
| *L. gibba* | 94.0 | 6.82 | 1.82 | --- | 2.79 | 10.5 | 13.8 | 2.4 | 30.7 | 42.6 |
| M | 92.0 | 5.84 | 1.42 | --- | 2.78 | 9.7 | 9.3 | 2.0 | 42.6 | 36.5 |
| 3/22/77 | | | | | | | | | | |
| *L. gibba* (covered) | 94.0 | 5.70 | --- | --- | --- | 10.4 | 21.8 | 7.5 | 22.5 | 35.6 |
| *L. gibba* (non-covered) | 94.0 | 5.67 | --- | --- | --- | 11.2 | 20.3 | 7.3 | 23.4 | 35.4 |
| M | 92.5 | 5.50 | --- | --- | --- | 10.9 | 20.9 | 7.2 | 24.4 | 34.3 |

[a] Composite samples.

[b] Nitrogen free extract.

[c] TKN x 6.25.

TABLE 64. WATER QUALITY OF STATIC AND AERATED[a] TEST CHANNELS SUPPORTING A MIXTURE OF *S. oligorhiza* AND *L. gibba*, WINTER, 1977

| Sample date | | mg/1 | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | $PO_4$ | K | Ca | $O_2$ | pH |
| 2/10/77 | Aerated[b] | 33.2 | 20.3 | 39.4 | 28.5 | 4.7 | 7.8 |
| | Static[c] | 37.0 | 18.6 | 37.9 | 30.9 | 7.4 | 7.7 |
| 2/15/77 | Aerated | 29.3 | 21.0 | 39.5 | 26.5 | 4.5 | 7.7 |
| | Static | 32.0 | 18.6 | 36.2 | 28.1 | 0.6 | 7.6-7.7 |
| 2/25/77 | Aerated | 26.4 | 18.3 | 39.2 | 23.5 | 4.6 | 7.7 |
| | Static | 25.9 | 17.1 | 36.0 | 26.8 | 0.4 | 7.7 |
| 3/2/77 | Aerated | 21.4 | --- | 40.6 | 30.0 | --- | 7.7 |
| | Static | 21.5 | --- | 35.9 | 30.7 | --- | 7.7 |
| 3/16/77 | Aerated | 9.7 | 9.3 | 31.9 | 23.3 | --- | 7.5 |
| | Static | 11.8 | 6.7 | 28.1 | 24.2 | --- | 7.2 |
| 3/31/77 | Aerated | 8.4 | 8.8 | 27.9 | 24.5 | --- | 7.8 |
| | Static | 8.2 | 6.5 | 24.2 | 27.3 | --- | 7.5-7.9 |
| % reduction | Aerated | 74.7 | 56.7 | 29.2 | 14.0 | --- | --- |
| | Static | 77.8 | 65.1 | 35.4 | 11.7 | --- | --- |

[a] Pump failure on February 25 terminated aerated vs. static comparisons. Separate values are shown however throughout the test.

[b] Data from one channel.

[c] Mean of two channels, excluding pH which shows actual values.

TABLE 65. WATER QUALITY OF STATIC AND AERATED[a] CONTROL CHANNELS, WINTER, 1977

| Sample date | | mg/1 | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | $PO_4$ | K | Ca | $O_2$ | pH |
| 2/10/77 | Aerated[b] | 33.5 | 17.8 | 35.1 | 31.8 | 6.6 | 8.0 |
| | Static[c] | 40.0 | 21.2 | 42.3 | 31.9 | 7.8 | 7.8–8.3 |
| 2/15/77 | Aerated | 31.2 | 18.0 | 37.9 | 31.3 | 5.8 | 7.9 |
| | Static | 33.9 | 21.8 | 42.2 | 30.2 | 1.6 | 7.8–8.0 |
| 2/25/77 | Aerated | 29.3 | 17.0 | 37.7 | 31.1 | 4.9 | 7.9 |
| | Static | 28.1 | 21.3 | 42.3 | 28.1 | 0.7 | 7.7 |
| 3/2/77 | Aerated | 26.2 | 16.0 | 38.0 | 31.3 | --- | 7.8 |
| | Static | 23.7 | 21.8 | 44.0 | 32.9 | --- | 7.6–7.8 |
| 3/16/77 | Aerated | 16.5 | 6.8 | 31.2 | 25.3 | --- | 7.8 |
| | Static | 13.9 | 14.8 | 36.1 | 25.7 | --- | 7.2–7.4 |
| 3/31/77 | Aerated | 9.7 | 6.4 | 29.5 | 26.9 | --- | 7.8 |
| | Static | 12.9 | 13.0 | 36.9 | 28.8 | --- | 7.8 |
| % reduction | Aerated | 71.0 | 64.4 | 22.4 | 15.4 | --- | --- |
| | Static | 67.8 | 40.4 | 16.1 | 12.5 | --- | --- |

---

[a] Pump failure on February 25 terminated aerated vs. static comparisons. Separate values are shown however throughout the test.

[b] Data from one channel.

[c] Mean of two channels, excluding pH which shows actual values.

The reduction rates for TKN and $PO_4$ were rapid through March 16 until the values dropped below 10 mg/l. Thereafter the rate of decline slowed, indicating a need to develop management techniques if water quality is to be further improved without an excessive retention time. It is possible that the duckweed system should be replaced by other biological treatment techniques when the TKN drops below 10 mg/l.

TABLES 66 and 67 show changes in BOD, COD, TR, VR, FR, and TSM for the mixed species and control channels. Similar ending values were noted for test and control channels. Throughout the total study we detected little differences between the duckweed and control channels for the above parameters. Under a well managed system where loading rates are controlled, and a high percent of soluble components are added and insoluble materials minimized, the duckweed lagoons may show improved performance over conventional lagoons. It is clear that under the conditions of the various tests, duckweeds are not detrimental to waste water renovation.

Standard plate counts showed little variation throughout Test 8 between test and controls (TABLES 68 and 69) and were similar to previous studies. Fecal counts in the test channels were less than in the controls. The reduced decline in bacterial counts over previous tests was due to the cooler temperatures.

Comparison of lagoon water quality from L. gibba covered and non-covered test channels showed that TKN and K reductions were greatest in channels under cover while Ca and $PO_4$ reductions were greatest in the non-covered channels (TABLES 70 and 71). Partial aeration (through February 25) appeared, again, to offer no advantage over static conditions, at least under the conditions of this and previous tests.

The non-covered controls (TABLE 65) and L. gibba (TABLE 71) showed similar water quality, while the covered L. gibba channels (TABLE 70) showed greater reductions than controls except for calcium. This suggested that L. gibba, when given protection from winter conditions, might be responsible for the water quality improvement as compared to the non-covered controls. However, growth of the plant under the two conditions was equal (TABLE 62). Therefore the better water treatment in the covered channels is best explained by the higher (though only 2 to 3°C) water temperatures and presumably the greater bacterial activity.

Compared to previous studies, reductions in $PO_4$, K, and Ca were surprisingly high. The reason is unclear at this time.

TABLES 72 and 73 show that BOD, COD, and other values in the covered test channels were similar to the non-covered test channels and controls (TABLE 67). The protection from ambient winter conditions was apparently insufficient to induce temperature-related differences in treatment.

Standard plate counts were similar in numbers and rate of decline (TABLES 74 and 75). However, fecal bacteria were initially lower in the covered channels and increased, while in the non-covered channels the reverse

TABLE 66. BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM), OF COMPOSITE SAMPLES FROM MIXED SPECIES[a] IN STATIC[b] TEST CHANNELS, WINTER, 1977

| Date | BOD | COD | TR | VR | FR | TSM |
|---|---|---|---|---|---|---|
| | mg/l | | | | | |
| 2/10/77 | 43 | 176 | 795 | 624 | 171 | 88 |
| 2/24/77 | 42 | 138 | 685 | 376 | 309 | 32 |
| 3/3/77 | -- | 125 | 728 | 447 | 281 | 47 |
| 3/7/77 | -- | 139 | 762 | 463 | 299 | 104 |
| 3/16/77 | 23 | 106 | 551 | 302 | 249 | 26 |

[a] _S_. _oligorhiza_ and _L_. _gibba_.

[b] Average of two channels.

TABLE 67. BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE[a] SAMPLES FROM STATIC CHANNELS, WINTER, 1977

| Date | BOD | COD | TR | VR | FR | TSM |
|---|---|---|---|---|---|---|
| | mg/l | | | | | |
| 2/10/77 | 39 | 168 | 783 | 628 | 155 | 104 |
| 2/24/77 | 30 | 166 | 777 | 571 | 206 | 40 |
| 3/3/77 | -- | 115 | 683 | 486 | 196 | 51 |
| 3/7/77 | -- | 146 | 701 | 490 | 211 | 100 |
| 3/16/77 | 21 | 98 | 546 | 279 | 267 | 24 |

[a] Average of two channels.

TABLE 68. STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF THE MIXED SPECIES[a] IN STATIC[b] TEST CHANNELS, WINTER, 1977

| Date | SPC/ml X $10^5$ | FC/100 ml X $10^6$ | FS/100 ml X $10^6$ |
|---|---|---|---|
| 2/10/77 | 1.6 | 4.7 | 3.1 |
| 2/24/77 | 2.1 | 5.8 | 4.3 |
| 3/3/77 | 2.2 | 6.3 | 5.6 |
| 3/7/77 | 2.1 | 5.9 | 5.1 |
| 3/16/77 | 1.8 | 4.9 | 4.7 |

[a] _S_. _oligorhiza_ and _L_. _gibba_.

[b] Average of two channels.

TABLE 69. STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE[a] SAMPLES FROM CONTROL CHANNELS, WINTER, 1977

| Date | SPC/ml X $10^5$ | FC/100 ml X $10^6$ | FS/100 ml X $10^6$ |
|---|---|---|---|
| 2/10/77 | 2.6 | 4.7 | 3.7 |
| 2/24/77 | 2.1 | 6.3 | 5.7 |
| 3/3/77 | 2.4 | 7.6 | 6.9 |
| 3/7/77 | 1.9 | 5.4 | 5.3 |
| 3/16/77 | 1.8 | 5.7 | 5.1 |

[a] Average of two channels.

TABLE 70. WATER QUALITY OF COVERED, AERATED AND STATIC TEST CHANNELS SUPPORTING L. gibba, WINTER, 1977

| Sample date | | mg/1 | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | $PO_4$ | K | Ca | $O_2$ | pH |
| 2/10/77 | Aerated[a] | 35.9 | 18.8 | 35.6 | 30.4 | 5.7 | 7.9 |
| | Static[b] | 37.4 | 19.4 | 37.2 | 31.9 | 6.5 | 7.7–7.8 |
| 2/15/77 | Aerated | 33.0 | 19.0 | 41.8 | 32.0 | 2.9 | 7.9 |
| | Static | 33.5 | 19.6 | 40.8 | 28.9 | 0.5 | 7.5–7.8 |
| 2/25/77 | Aerated | 29.6 | 18.0 | 35.8 | 30.4 | 3.7 | 7.8 |
| | Static | 28.9 | 17.0 | 38.0 | 24.8 | 0.6 | 7.7 |
| 3/2/77 | Aerated | 24.5 | 17.3 | 39.1 | 31.0 | --- | 7.8 |
| | Static | 24.2 | 14.8 | 38.7 | 31.7 | --- | 7.7 |
| 3/16/77 | Aerated | 12.5 | 8.6 | 34.3 | 28.5 | --- | 7.4 |
| | Static | 11.5 | 8.2 | 31.7 | 27.3 | --- | 7.2–7.4 |
| 3/31/77 | Aerated | 7.6 | 8.0 | 29.8 | 29.6 | --- | 7.8 |
| | Static | 8.0 | 7.8 | 26.8 | 28.7 | --- | 7.5 |
| % reduction | Aerated | 78.8 | 57.9 | 28.7 | 7.5 | --- | --- |
| | Static | 78.6 | 60.2 | 34.3 | 10.0 | --- | --- |

[a] Aerated, data from one channel. Pump failure on February 25 terminated aerated vs. static comparisons.

[b] Static, mean of 2 channels excluding pH.

TABLE 71. WATER QUALITY OF NON-COVERED, AERATED AND STATIC TEST CHANNELS SUPPORTING L. gibba, WINTER, 1977

| Sample date | | mg/l | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | $PO_4$ | K | Ca | $O_2$ | pH |
| 2/10/77 | Aerated[a] | 32.6 | 18.3 | 33.6 | 30.6 | 5.8 | 7.9 |
| | Static[b] | 32.9 | 17.3 | 32.5 | 30.7 | 6.7 | 7.8-8.1 |
| 2/15/77 | Aerated | 30.5 | 18.3 | 36.1 | 29.4 | 4.4 | 7.9 |
| | Static | 29.9 | 17.4 | 34.9 | 29.5 | 0.8 | 7.7-8.0 |
| 2/25/77 | Aerated | 28.4 | 16.4 | 35.2 | 27.8 | 4.7 | 7.8 |
| | Static | 25.6 | 15.1 | 33.0 | 27.7 | 0.6 | 7.7-7.9 |
| 3/2/77 | Aerated | 25.8 | 15.8 | 37.6 | 31.9 | --- | 7.7 |
| | Static | 21.6 | 13.8 | 33.5 | 29.2 | --- | 7.7-7.9 |
| 3/16/77 | Aerated | 15.8 | 7.1 | 31.4 | 25.8 | --- | 7.4 |
| | Static | 14.2 | 5.5 | 28.3 | 24.6 | --- | 7.2-7.4 |
| 3/31/77 | Aerated | 10.3 | 6.8 | 31.6 | 26.8 | --- | 7.8 |
| | Static | 9.2 | 5.2 | 28.8 | 25.0 | --- | 7.5-7.6 |
| % reduction | Aerated | 68.4 | 62.8 | 15.9 | 15.9 | --- | --- |
| | Static | 72.0 | 70.1 | 17.5 | 18.6 | --- | --- |

[a] Aerated, data from one channel. Pump failure on February 25 terminated aerated vs. static comparisons.

[b] Static, mean of 2 channels excluding pH.

TABLE 72. BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM) OF COMPOSITE[a] SAMPLES FROM L. gibba STATIC, COVERED TEST CHANNELS, WINTER, 1977

| Date | BOD | COD | TR | VR | FR | TSM |
|---|---|---|---|---|---|---|
| | mg/1 | | | | | |
| 2/10/77 | 42 | 176 | 867 | 666 | 201 | 80 |
| 2/24/77 | 18 | 95 | 500 | 335 | 165 | 36 |
| 3/3/77 | -- | 123 | 731 | 440 | 291 | 43 |
| 3/7/77 | -- | 147 | 782 | 476 | 306 | 88 |
| 3/16/77 | 21 | 96 | 526 | 284 | 242 | 28 |

[a] Average of two channels.

TABLE 73. BIOCHEMICAL OXYGEN DEMAND (BOD), CHEMICAL OXYGEN DEMAND (COD), TOTAL RESIDUE (TR), VOLATILE RESIDUE (VR), FIXED RESIDUE (FR), AND TOTAL SUSPENDED MATTER (TSM), OF COMPOSITE[a] SAMPLES FROM L. gibba STATIC, NON-COVERED TEST CHANNELS, WINTER, 1977

| Date | BOD | COD | TR | VR | FR | TSM |
|---|---|---|---|---|---|---|
| | mg/1 | | | | | |
| 2/10/77 | 46 | 216 | 884 | 736 | 148 | 88 |
| 2/24/77 | 18 | 115 | 717 | 350 | 367 | 22 |
| 3/3/77 | -- | 117 | 720 | 403 | 317 | 39 |
| 3/7/77 | -- | 132 | 743 | 501 | 242 | 118 |
| 3/16/77 | 23 | 101 | 548 | 280 | 268 | 34 |

[a] Average of two channels.

TABLE 74. STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE[a] SAMPLES FROM L. *gibba* STATIC, COVERED CHANNELS, WINTER, 1977

| Date | SPC/ml X $10^5$ | FC/100 ml X $10^6$ | FS/100 ml X $10^6$ |
|---|---|---|---|
| 2/10/77 | 2.6 | 4.4 | 3.6 |
| 2/24/77 | 1.9 | 4.3 | 3.8 |
| 3/3/77 | 2.4 | 5.1 | 4.2 |
| 3/7/77 | 1.9 | 4.1 | 3.7 |
| 3/16/77 | 2.1 | 4.9 | 4.1 |

[a] Average of two channels.

TABLE 75. STANDARD PLATE COUNT, FECAL COLIFORM, AND FECAL STREPTOCOCCI COUNTS OF COMPOSITE[a] SAMPLES FROM L. *gibba* STATIC, NON-COVERED TEST CHANNELS, WINTER, 1977

| Date | SPC/ml X $10^5$ | FC/100 ml X $10^6$ | FS/100 ml X $10^6$ |
|---|---|---|---|
| 2/10/77 | 2.6 | 8.0 | 7.9 |
| 2/24/77 | 1.8 | 6.3 | 5.9 |
| 3/3/77 | 2.0 | 6.1 | 5.9 |
| 3/7/77 | 1.9 | 5.4 | 5.3 |
| 3/16/77 | 2.2 | 7.0 | 6.8 |

[a] Average of two channels.

occurred. The bacterial increase under the covered channels suggests that the TKN values showed greater decline in the covered channels due to the slightly higher temperatures which could have enhanced bacterial action.

### Test 9, Water Quality of Static Control Channels and Duckweed Covered Aerated Channels, Summer, 1977

This test was conducted to evaluate growth of a mixture *S. oligorhiza* and *L. gibba* aerated under summer conditions and to compare water quality changes of duckweed aerated channels with static controls. Aeration under summer conditions had not been tested. No differences were detected between aeration and static conditions during the winter months.

Six text channels were used for this study. All channels were flooded with water from the anaerobic lagoon to raise nutrient levels prior to plant stocking. On June 17, 1977 three channels were seeded with 9.09 kg of the duckweed mixture and aeration was initiated. Three channels (without duckweed) remained static and served as controls. Harvesting began on July 3 when channels were covered with the duckweed.

Chemical analysis of lagoon water in previous tests indicated that TKN and $PO_4$ were the best indicators of treatment. Oxygen, also useful, was measured through the study primarily to indicate whether or not the lagoons were aerobic. $NO_3$ and $NO_2$ were monitored to determine if nitrification was active.

TABLE 76 shows that TKN reduction in the aerated duckweed channels (87%) was greater than the static control (76.8%). However, during the first 24 days TKN reduction in the aerated channel was 85% and the static 67%. In the last 20 days of the study the aerated channel showed 13% reduction compared to 30% for the static channel. The slower decline in the test channels the last 20 days may be explained by the oxidation of the more difficult to degrade organic compounds, and thus replacing TKN being removed by the duckweed. In the static channels, the more rapid decline the last 20 days may be explained by the settling of organic compounds, and the possible absence of a nitrification-denitrification sequence. Due to the low oxygen and with a pH of less than 10, $NH_4^+$ should form and stay in solution. Without nitrifying and denitrifying bacteria the conversion of $NH_4$ ions to nitrogen gas is greatly reduced. Settling of organic material offers a feasible explanation for the control channels in the absence of a nitrification-denitrification sequence.

During the first 24 days TKN reduction in the aerated channels averaged 1.53 mg/l/day. Uptake by the duckweed accounted for .162 mg/l/day or 11% of the TKN. Dissolved oxygen levels were high enough to permit nitrification to occur. Undoubtedly less oxygen was present in the lower portions of the channel, and denitrification could occur, resulting in the loss of nitrogen as a gas. However, during the last 20 days, oxygen was quite high, and denitrification may not have followed nitrification. The only way significant nitrogen could be lost from the system would be through uptake by duckweed which averaged .141 mg/l/day. The nitrogen reduction was

TABLE 76. WATER QUALITY[a] OF DUCKWEED COVERED TEST (AERATED) AND CONTROL (STATIC) CHANNELS, SUMMER, 1977

| Sample date | | mg/l | | | | | |
|---|---|---|---|---|---|---|---|
| | | TKN | $NO_3$ | $NO_2$ | $PO_4$ | DO[b] | pH[c] |
| 6/25/77 | Aerated[d] | 43.1 | <.5 | <.25 | 31.9 | .9 | 7.5-7.6 |
| | Static | 45.6 | <.5 | <.25 | 31.7 | 0 | 7.6-7.7 |
| 7/2/77 | Aerated | 33.5 | <.5 | <.25 | 30.6 | .8 | 7.5-7.6 |
| | Static | 35.7 | <.5 | <.25 | 32.2 | 0 | 7.6-7.9 |
| 7/9/77 | Aerated | 22.3 | <.5 | <.25 | 29.4 | .9 | 7.4-7.5 |
| | Static | 26.9 | <.5 | <.25 | 32.0 | .1 | 7.8-7.9 |
| 7/13/77 | Aerated | 13.5 | <1.4 | <.50 | 30.7 | 1.0 | 7.4-7.5 |
| | Static | 20.2 | <.5 | <.25 | 32.4 | .2 | 7.9-8.0 |
| 7/19/77 | Aerated | 6.4 | <2.0 | <.90 | 24.3 | 2.5 | 7.4-7.7 |
| | Static | 15.2 | <.5 | <.25 | 32.2 | .7 | 7.8-8.0 |
| 7/27/77 | Aerated | 6.6 | <.5 | <1.60 | 31.8 | 3.1 | 7.4-7.6 |
| | Static | 14.2 | <.5 | <.25 | 24.6 | 0 | 7.8-8.0 |
| 8/2/77 | Aerated | 6.5 | <.5 | <.90 | 29.5 | 2.9 | 7.4-7.5 |
| | Static | 12.1 | <.5 | <.25 | 23.5 | 0 | 7.9-8.1 |
| 8/8/77 | Aerated | 5.6 | <.5 | <.50 | 30.0 | --- | 7.4 |
| | Static | 10.6 | <.5 | <.25 | 24.1 | --- | 7.9-8.1 |
| % reduction | Aerated | 87.0 | --- | --- | 5.9 | --- | --- |
| | Static | 76.8 | --- | --- | 23.9 | --- | --- |

[a] Means of 3 channels, water temperature 28 ± .5°C.
[b] Taken between 1200-1400 hour.
[c] Range of 3 channels.
[d] Covered with a mixture of L. gibba and S. oligorhiza; static controls without duckweed.

only .04 mg/l/day. Nitrogen was probably being moved into the water column from the sludge zone to replace that removed by the duckweed.

Nitrogen conservation may best be achieved by maintaining a complete aerobic system, or anaerobic system with a pH of less than 10. A facultative system in which nitrification-denitrification can occur, will result in nitrogen loss, which is acceptable for a waste treatment scheme, but undesirable for waste management, in which maximum utilization of nutrients are desired.

Under the static system, phosphorus reduction (23.9%) exceeded the aerated system (5.9%). Duckweeds do not remove high quantities of phosphorus, and the aeration most likely kept phosphorus somewhat suspended in the water column. The low reduction in phosphorus was seen throughout this project and removal by duckweeds was not very effective. The development of clones capable of concentrating phosphorus is desirable, or alternate management schemes must be developed.

The pH of the static lagoons in this test, and others, has been conducive to maintaining nitrogen within the system as $NH_4^+$. Thus the opportunity for extraction for use is optimized. If removal only is desired, ammonification or a nitrification-denitrification sequence would be needed.

TABLE 77 shows the yield of duckweed on the aerated system. The yield was one of the lowest recorded, and corresponded only with winter yields. During the summer of 1976 we also had yields lower than spring and fall studies, but the 1976 summer yields under static conditions were over 3 fold the 1977 summer yield.

It is interesting to note that higher yields were obtained when the test channels were near anaerobic conditions (up to July 19). Thereafter the yield declined. It appears that the duckweed we studied may give best growth under anaerobic conditions. Previous tests indicated that _S. oligorhiza_ and _L. gibba_ had highest growth rates in cooler months. _S. polyrhiza_, which performed best in summer temperatures, may have been a more desirable plant. The placement of aerators in the duckweed channels created open areas where the air surfaced. Even though this area was small, it increased crowding of the duckweed and may have reduced growth. Further studies may be warranted to evaluate duckweed growth and waste treamtnet under aerobic conditions.

Chemical analysis of the duckweed (TABLE 78) agrees with previous tests. The low TKN values were expected on the low nutrient system. The high K levels can be accounted for by the presence of _L. gibba_. It is not known if aeration reduced the TKN uptake. With the reduced growth rate N utilization may have diminished.

TABLE 77. YIELD OF A MIXTURE OF *L. gibba* AND *S. oligorhiza* UNDER AERATION IN STATIC TEST CHANNELS FLOODED WITH DAIRY WASTE, SUMMER, 1977

| | Harvest date[a] | | | | Total/44 days | Harvest/mo[c] | |
|---|---|---|---|---|---|---|---|
| | 7/3 | 7/19 | 7/26 | 8/1 | | kg/ha | lbs/acre |
| kg harvested/$m^2$[b] | | | | | | | |
| wet | .582 | .655 | .455 | .473 | 2.165 | --- | --- |
| dry (6.8%) | .040 | .045 | .031 | .032 | .147 | 405 | 361 |

[a] Means of 3 test channels.

[b] Mean biomass removed from ½$m^2$.

[c] Total harvest values are based on one-half hectare or acre being harvested. One-half remains for regrowth.

TABLE 78. CHEMICAL COMPOSITION OF A MIXTURE OF *S. oligorhiza* AND *L. gibba* UNDER LOW NUTRIENT AND AERATED CONDITIONS, SUMMER, 1977

| Sample date | % of dry matter[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TKN | P | K | Ca | Fiber | Ash | Fat | NFE | Crude protein[b] |
| 7/19/77 | 5.33 | 1.12 | 3.30 | 1.57 | 10.0 | 15.1 | 6.7 | 27.4 | 33.3 |
| 7/25/77 | 5.15 | 1.03 | 3.42 | 1.41 | 8.9 | 12.9 | 6.9 | 37.5 | 32.2 |
| 8/1/77 | 5.23 | 1.04 | 3.39 | 1.67 | 8.0 | 14.2 | 5.5 | 35.9 | 32.7 |
| 8/8/77 | 5.31 | 1.10 | 4.13 | 1.65 | 8.1 | 15.1 | 6.9 | 33.2 | 33.2 |
| 8/15/77 | 4.59 | 1.01 | 4.40 | 1.64 | 9.3 | 15.1 | 6.6 | 34.5 | 28.7 |

[a] Mean of 3 channels.

[b] TKN X 6.25.

## PRELIMINARY FEEDING TRIALS

### Palatability

S. polyrhiza and S. oligorhiza were presented to young dairy cows (204-227 kg). The plants were grown on dairy waste lagoons, harvested, rinsed and presented wet or dry alone or in combination with a feed concentrate (corn meal). Duckweed ranged from 0 (controls) to 100% of the diet.

The duckweed was palatable whether or not it was mixed with a feed concentrate. However, at the 100% duckweed diet the cows were reluctant to feed on it unless washed. Animals consumed up to 75% duckweed ration readily for a period of 26 days and gained in weight. The animals on the diet showed no external signs of health disorders such as extreme weight loss, loss of appetite, diarrhea, or nervousness.

### Milk Quality

In a preliminary test to determine if any serious problems were evident with milk quality from cows fed duckweed, a single lactating cow was given a diet containing a combination of duckweeds (ca 25% of diet on dry weight basis) for 7 days. No significant change in milk flavor other than a possible increase in fat acid degree value was detected.

### Digestibility

Separate "in vitro" digestibility studies with S. oligorhiza, S. polyrhiza, and L. gibba, showed digestibility of about 70%. This is within the range of current rations. Wolffia sp. showed "in vitro" digestibility of 97%. This value exceeds typical cattle feeds, suggesting a high quality of feed. Further nutritional research is needed before duckweeds can be recommended as a feed source.

# SECTION 8

# DISCUSSION

## SPECIES COMPOSITION

As noted in Tests 5, 6, and 8, mixed culture treatments exhibited no significantly different yields than treatments containing one species or clone. It is a broadly accepted dogma of ecologists that diversity promotes stability. The utilization of mixed cultures of several species and/or clones has two potential advantages. Seasonal dominance of one or more clones would alter the relative composition of the plant biomass. This would tend to even out yield over the entire year and allow a more reliable feed supply-utilization scheme, perhaps lessening the need for storage.

The second potential advantage of mixed cultures is the stability expected against pests and/or disease. Pests of duckweeds are few, but Scotland (1934; 1940) discusses and reviews obligate and facultative relationships between aquatic insects and species of Lemna. Since clones inherently have only small amounts of genetic variability, an infestation or disease could have devastating and rapid effects on the plant stand. Polyclonal cultures would theoretically be less susceptible to such hazards. Though few diseases or pests of duckweeds are known, it is important to recognize the possibility of their existence, and to manage duckweed cultures accordingly.

## ANNUAL AND SEASONAL YIELD

Utilizing yield estimates calculated for each experiment two estimates of total annual yield (dry weight) per hectare were determined. One estimate was based on mean yield (irrespective of species and treatment) as shown in TABLE 79. The second estimate was based on the highest yield during each 3-month period (also irrespective of species and treatments). Yield was estimated to be 17,577 kg/ha/yr (mean) and 22,023 kg/ha/yr (high). The former value indicates the average yield that would have been produced annually if grown on a one hectare lagoon. The latter value is an estimate of the best annual yield obtained (for the prevailing conditions during the study) on a one hectare basis.

Seasonal variation of yield (mean) was dramatic. Winter yield was approximately 25% of fall yield while spring and summer yields were approximately 75% of the yield during the fall months. This variation could be decreased if duckweed clones are found to be optimally suited to each season, thus stabilizing supply for the utilization of the plants.

### TABLE 79. ESTIMATED ANNUAL DRY WEIGHT YIELD PER HECTARE OF DUCKWEEDS PRODUCED ON LAGOONS RECEIVING DAIRY CATTLE WASTES, 1976-77[a]

| | | Tri-monthly basis | |
|---|---|---|---|
| | | mean yield | highest yield (single estimate) |
| Yield May - July | | 1663 kg/mo<br>X 3 months | 2215 kg/mo<br>X 3 months |
| | subtotal | 4989 kg | 6645 kg |
| Yield Aug. - Oct. | | 2122 kg/mo<br>X 3 months | 2143 kg/mo<br>X 3 months |
| | subtotal | 6366 kg | 6429 kg |
| Yeild Nov. - Jan. | | 518 kg/mo<br>X 3 months | 761 kg/mo<br>X 3 months |
| | subtotal | 1554 kg | 2283 kg |
| Yield Feb. - April | | 1556 kg/mo<br>X 3 months | 2222 kg/mo<br>X 3 months |
| | subtotal | 4668 kg | 6666 kg |
| | Total[b] | 17,577 kg/ha/yr | 22,023 kg/ha/yr |

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[a] Values are irrespective of species and treatment.

[b] To obtain lb/ac/yr multiply by 0.893.

Further research should include plant screening and selective breeding studies to obtain clones which are adapted (seasonally) to remove or reduce the variation of yield.

Based on the mean chemical content of the plants; 1,019 kg N, 248 kg P, and 353 kg K would have been removed via harvest from one hectare. Each 454 kg cow excretes 0.18 kg of N, 0.02 kg of P, and 0.11 kg of K daily (Loehr, 1969). Based on these values, the duckweeds harvested per hectare effectively removed N of 15.5 cows, P of 34.0 cows, and K of 8.8 cows. It should be noted that the lagoon system was overloaded during the study and the dynamics of nutrient removal under lower nutrient concentrations may differ substantially from this.

Though duckweeds in this system were grown on cattle wastes, growth has been demonstrated on swine wastes (Culley and Epps, 1973; Stanley and Madewell, 1975) and should be applicable to other animal waste lagoon systems. Duckweeds may find application as part of municipal, chemical plant, and food processing plant waste treatment systems as well.

## NUTRIENT PLASTICITY

Duckweeds show the ability to increase dry weight percentages of nutrients when placed on waters containing high concentrations of nutrients or upon periodic reflooding. An optimum dilution rate for growth of L. minor on swine wastes was determined by Stanley and Madewell (1975). It appears that separate optima for growth and nutrient content of duckweed clones are likely to exist. Also, it is probable that there exists an optimum range for water rennovation efficiency. During the study, growth rate did not seem to decline until TKN of the water was below ca 20 mg/l. To validate this statistically simultaneous studies need to be performed under different nutrient regimes. This was unfortunately obviated by physical limitations of the lagoon system in this study.

Further research involving long term growth and nutrient content studies in relation to water chemistry are needed, as this system frequently was operated at overloaded conditions and in an unusually low rainfall year. Clearly, if stable economic benefits are to be derived from the waste rennovation system, lagoon management schemes must attempt to optimize: (1) plant nutrient content, (2) growth, and (3) water rennovation efficiency. Thus, further study is needed to delineate optimum concentrations of bovine wastes in relation to the above criteria.

## POTENTIAL ECONOMIC BENEFITS

As stated by Wolverton, Barlow, and McDonald (1975) and NAS (1976), aquatic plants are potentially useful as feed, energy, and fertilizer sources. If one of these potentials could be exploited, utilization of a waste treatment system for producing aquatic plant crops might prove economical to agricultural operations and improve waste treatment.

Nutrient content of unprocessed duckweeds compare favorably with processed high protein crops, used as livestock feed protein supplements, as shown in TABLE 80. Mean crude protein content during this study (irrespective of species and treatments) was 36%. Duckweeds, however, have been shown to contain 40 to 45% (dry weight) crude protein (Truax et al., 1972; Culley and Epps, 1973). This indicates the potential value of duckweeds as a protein supplement (NAS, 1971). As a feed for cattle duckweeds have been shown to be palatable. The only potentially harmful nutrient, observed in this study, was the potassium content of L. gibba (4.2%) which could possibly upset the cation balance in dairy cattle. The concentration may be compensated for by "dilution" with other plant materials containing lower amounts of potassium. Some duckweeds contain calcium oxalate crystals but it is not detrimental to cattle.

Yield of duckweeds exceeds those of the high protein crops shown in TABLE 80. Yield estimates (high and mean) of duckweeds are from TABLE 79. Multiplying yield (kg/ha) by the percentage of crude protein represents the total protein yield (TPY). From these values, hectare equivalents were determined by dividing TPY for each crop into the TPY of duckweeds. Thus, for example, one hectare of duckweed would produce the equivalent protein of 26 and 32 hectares of cotton (seed, mech-extd.), using the mean and high duckweed estimates respectively.

The economic feasibility of using duckweeds as an ingredient in a protein supplement for dairy cattle was determined by using Feedmix, a computer program (Just et al., 1968 as modified by Pope). Various feed sources (including those shown in TABLE 80) were entered at fixed prices (average prices for the first six months of 1977) and duckweed was entered at variable price. Results indicate that the value of duckweed would vary between $3.50 and $5.57 per hundred weight depending on the particular formulation. For example, one possible formulation containing 18.69% duckweed would be economically feasible if the cost of duckweed did not exceed $3.79 per hundred weight. Thus, cost of production, transportation, and processing of duckweeds could not exceed $3.79 per hundred weight for this particular formulation to be economical. Considering the yield per hectare and the close proximity of the plant source to the point of consumption; if on site processing was feasible, the above price constraints were met, and the total yield (high) were utilized as protein supplement ingredients for this particular ration, the producer would save an estimated $745.38 per acre-year ($1841.09 per hectare-year).

Hromadka (1976) found that the predominant method of waste management for Louisiana dairymen was lagooning. For herds with greater than 100 head, he determined average investment cost for lagooning was $3,266.67 or $17.49 per head. If duckweeds are grown and utilized on a lagoon waste treatment system, it appears that the investment and operating costs could be recovered while still providing the necessary waste treatment, based on the above figures. It should be clear, however, that the economic efficiency of agriculture is due to the low cost of fossil fuels. United States agriculture is energy intensive and any increase in fuel costs will have an adverse impact on feed costs. Duckweeds may be a low energy cost crop, and may become an even more attractive alternative in the future.

TABLE 80. PARTIAL CHEMICAL COMPOSITION AND YIELD COMPARISONS OF SOME COMMON ANIMAL FEED PROTEIN SUPPLEMENTS[a] AND DUCKWEEDS

| Plant material | Yield[b] kg/ha | % dry weight | | | | | | | | Crude protein yield kg/ha | Hectare equivalents | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Crude protein | N | P | K | Ash | Fiber | Ca | Fat | | mean | high |
| Cotton, seed w some hulls mech-extd. grnd 5-01-617[c] | 448 | 44.7 | 7.2 | 1.18 | 1.35 | 6.6 | 11.8 | .20 | 6.0 | 200 | 32 | 40 |
| Soybean, seeds, solv-extd. 5-04-604 | 1263 | 52.4 | 8.4 | .73 | 2.15 | 6.6 | 5.9 | .33 | 1.3 | 662 | 10 | 12 |
| Peanut, kernels, solv-extd. 5-03-650 | 1792 | 51.8 | 8.3 | .71 | — | 4.9 | 14.3 | .22 | 1.3 | 928 | 7 | 9 |
| Corn, gluten w bran 5-02-903 | 1630 | 28.6 | 4.6 | .86 | .63 | 7.3 | 8.1 | .49 | 2.9 | 466 | 14 | 17 |
| Duckweeds[d] | 17577 (mean)<br>22023 (high) | 36 | 5.8 | 1.41 | 2.01 | 13.7 | 8.3 | 1.7 | 4.8 | 6328 (mean)<br>7928 (high) | 1 | 1 |

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[a] Source: Atlas of Nutr. Data on U.S. and Can. Feeds. NAS. Wash., D. C. 1971.

[b] All values except duckweed based on data from: Louisiana Crop Production, USDA, La. Crop and Livestock Reporting Service, Alexandria, La., 1975.

[c] National Academy of Science feed reference number.

[d] Means during this study irrespective of treatments and species.

## LAGOON MANAGEMENT

Although lagoon systems have been used in agricultural operations for many years, we located no research data concerning management of lagoons to optimize aquatic plant production. This study elucidated several points for future research in this regard.

Optimum water nutrient content for the use of bovine wastes in aquatic plant production is not known. As indicated earlier, separate optima of different clones in relation to growth, nutrient content, and water rennovation efficiency may exist. Polyclonal cultures will also need to be considered in further detail in regard to lagoon management. If efficiency of a waste treatment system utilizing aquatic plants is to be achieved, then comparative studies of lagoon management schemes are needed.

As shown in several of our experiments, duckweeds exhibited increased nutrient content when grown on high nutrient waters. Periodic addition of nutrients may prove valuable in producing a better quality plant for feed.

Covering duckweed channels during cold weather was shown to be partially effective; however, further work is needed. Screening of duckweeds and selective breeding to obtain cold-tolerant clones is needed. If winter growth could be increased, by obtaining cold-tolerant clones or increasing lagoon temperatures, water renovation efficiency could possibly be increased during the winter season.

## WATER QUALITY

Of the several chemical and physical parameters measured through this study, TKN and P appeared most useful in evaluating water quality. Phosphorus may be the best single measure of water quality due to its slow removal over time and its importance in contributing to eutrophication in receiving waters. The TKN, due to its value as a plant nutrient and rather consistent decline over time in all tests, also proved useful in evaluating water quality. However, the TKN value includes many nitrogen sources which vary considerably. While the total TKN may be declining, readily available plant nutrient components may be increasing, and stimulate plant growth in receiving waters. A measurement of the TKN alone would not elucidate the cause.

Phosphorus removal may or may not be accelerated in aerobic treatment as compared to anaerobic. Tables of Test 6 (49 to 51) indicated greater removal, but was refuted in Test 9 (TABLE 76).

There was a discrepancy between the rates of phosphorus decline we observed and the phosphorus removed by plant growth. Phosphorus content of the duckweeds ranged from 1.32% to 1.55% (dry weight) for the Test 6 study. Calculations based on plant harvest during this period show that the quantity of phosphorus removed as plant material would reduce orthophosphate concentration in the test channels by approximately 0.6 mg/1 (0.02 mg/1/day). This rate is considered low. Sutton and Ornes (1975) gave phosphorus rate at approximately 0.09 mg/l/day for a 28 day retention time in their work with a mixed culture of L. gibba and L. minor grown on static sewage effluent.

Phosphorus may have been removed by the sludge layer. Wells (1969) found that certain acclimated sludges were capable of removing phosphorus from waste waters at rates of 65 mg/l/hr. Wells also found that 50% of the phosphorus moved between the sludge layer and the water column. The reaction was oxygen dependent, but he was unable to characterize the sludge further. Such a mechanism may have been operative in our test channels. Minimizing the sludge zone would tend to keep phosphorus in solution and facilitate uptake by duckweeds. In our system, which had high loading rates, the sludge layer approached 15 cm at times. This layer could have accounted for the variation in percent phosphorus reduction between duckweed and control channels.

Removal of phosphorus from animal waste water systems may be the most difficult problem of all. We encountered greater than 30 mg/l phosphorus concentrations in the effluent. The possibility of obtaining a clone of duckweed that concentrates phosphorus cannot be ruled out. However, until a mechanism for phosphorus removal is developed for lagoon systems, the only realistic means of reducing phosphorus is an effluent is by decreasing loading rates.

Nitrogen uptake during plant growth accounted for only part of the nitrogen leaving the duckweed channels and did not explain nitrogen loss from control channels. Approximately 5.05 kg (dry matter) of S. oligorhiza/channel was harvested during the summer study. Based on a mean TKN content of 5.72%, approximately 0.29 kg of TKN was removed as plant material. When corrected for water loss of 16 cm, the concentration of TKN removed from the system was calculated to be 48.1 mg/l. Of the 48.1 mg TKN/l removed, 11.0 mg TKN/l or 23% was accounted for by plant harvest. Similar calculations showed nitrogen removed as plant material accounted for 10.4% and 19.8% of the total nitrogen reductions for the Test 6 and 7 studies respectively. Settling or organic matter was undoubtedly responsible for a part of the observed decline, but nitrification-denitrification processes may have been important since the system was facultative at times.

Other workers attributed nitrogen losses to volatilization of ammonia but ammonia stripping requires a pH near 10 and a high air/liquid ratio. The pH requirement is based on the equilibrium of molecular ammonia and ammonium ions in water and is represented by the following equation:

$$NH_4^+ + OH^- \rightarrow NH_3 + H_2O$$

Below a pH of 10, ammonium ion predominates and little stripping should occur. The pH of the test channels ranged from 7.0 to 8.5 for all studies.

Samples (1967) and Painter (1970) give the following conditions as favorable to nitrification:

| | |
|---|---|
| Dissolved Oxygen | > 0.5 mg/l |
| Temperature | 5° to 45°C |
| Detention Time | 8 hours |
| pH | 6.5 to 9.5 |
| BOD/VS | < 0.4 mg/l |

Previous data indicated that the temperature, detention time, pH, and BOD/VS ratio criteria were satisfied throughout the three studies. Oxygen levels were sufficient during part of both winter studies, and presence of phytoplanton blooms at and slightly below the surface, and the continued appearance of zooplankton populations indicated that the test channels were slightly aerobic in the upper few cm. Conditions for nitrification to occur, at least in the upper few cm, were probably satisfied. Thus complex nitrogen compounds could be degraded to ammonium ions and further oxidized to nitrites and nitrates.

Conversion of the nitrites and nitrates to nitrogen gas (denitrification) takes place under anaerobic and near-anaerobic (0.5 mg/l oxygen) conditions (Wheatland, Barrett, and Bruce, 1959). Nitrification-denitrification along a vertical oxygen gradient (as in our system) in a pig manure lagoon was, however, conclusively demonstrated by van Fassen and Dijk (1974), and Patrick et al., 1976 with flooded soils. Thus it was likely that some nitrogen loss occurred in our system through nitrification-denitrification, but we do not know if a significant amount was removed.

Potassium is seldom regarded as a pollutant and is generally overlooked in discussions of animal waste treatment operations. We monitored potassium since it is a plant nutrient and of vital importance in cattle feeds. Duckweeds remove potassium, and could affect the quantity of duckweeds blended with cattle rations. The slow decline of potassium was likely due to leaching from the sludge layer, as in laboratory tests rapid leaching of $PO_4$ from sludge was demonstrated.

The difference in calcium levels between lagoons results from different loading rates and soil content. The mean concentrations of calcium were 71.3 mg/l and 62.7 mg/l for test lagoons 1 and 2 respectively. Calcium levels varied in like manner for both duckweed channels and controls. We expected plant uptake to reduce calcium levels in the duckweed channels, but it was apparently offset by leaching from the soil and sludge layer.

Further analysis of data may show some useful relationships between TOC, TSM, TKN, COD, TR, and P in evaluating effluent quality. Should there be a fixed ratio between TOC, COD, TR, or TSM and TKN or P, then a single measurement may prove useful in monitoring waste water effluent involving macronutrients.

Total suspended solids was useful in indicating a potential settleable load, or a reduction in light penetration. However, it tells nothing of the associated nutrient load or nutrients in solution. The total residue includes both the volatile and fixed residue. There may be a useful ratio index between the TR and TKN or phosphorus. Further analysis is needed to make these determinations.

## BACTERIOLOGICAL IMPLICATIONS

Although considerable effort was placed on bacterial analysis in this study, it must be remembered that the system was overloaded with waste material. In evaluating the performance of duckweed at high nutrient levels, high concentrations of bacteria were introduced into the system. The declines observed were expected, as well as the high concentrations.

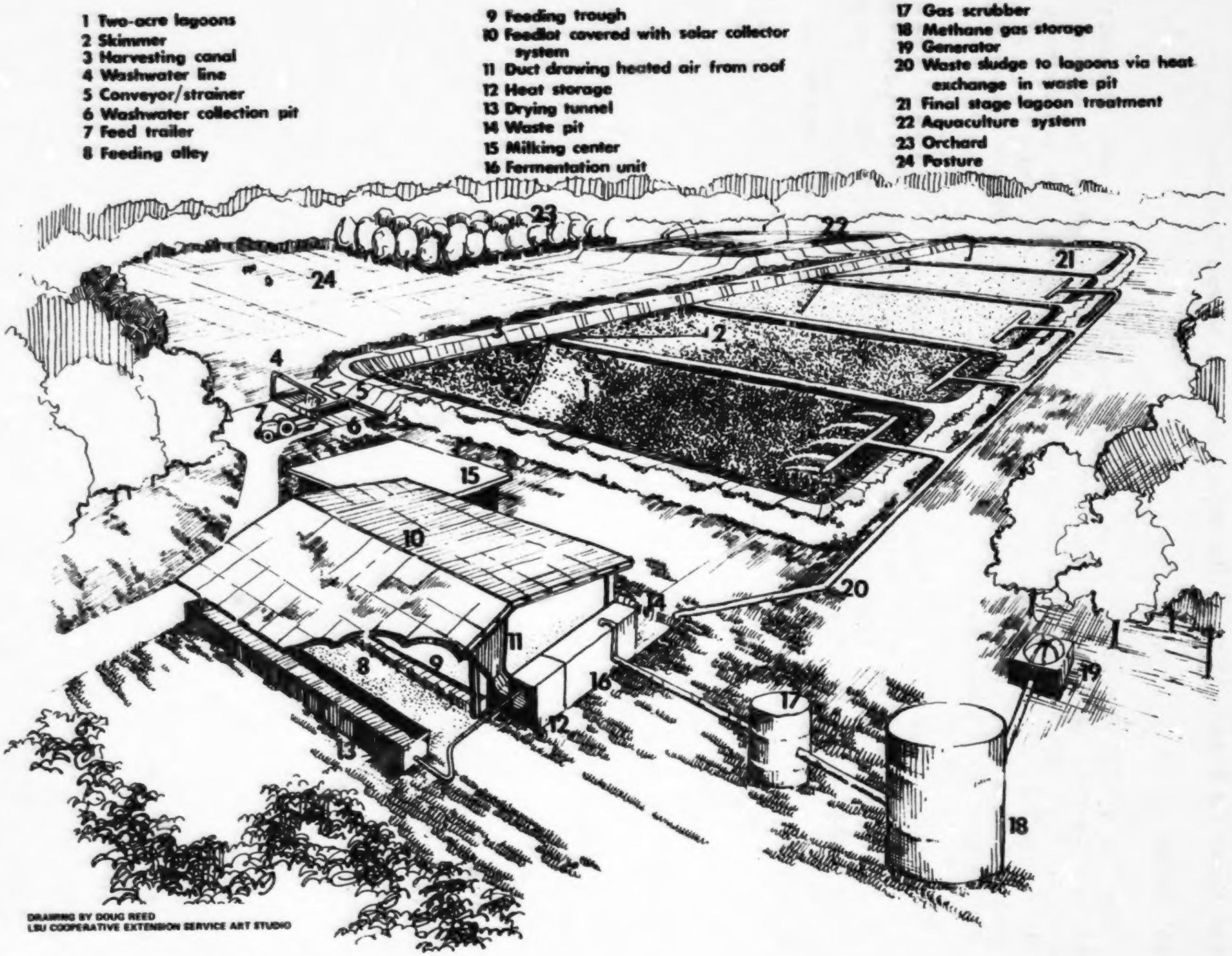
Although pathogenic bacteria were evident in the lagoons, they were confined primarily near the floor. Overflow from the lagoons was from surface waters associated with the duckweed. Pathogenic bacteria were not found in the effluent. In addition, studies on the palatability, growth, and milk quality failed to show evidence of disease transmission, though further studies are needed. When using duckweed as a feed ingredient, it was always washed with chlorinated water. This practice should be continued as a safety precaution.

## LAGOONS AND LAND APPLICATION

Throughout this study, it became clearly evident that land application of manure was labor intensive and expensive as compared to lagooning. In high rainfall areas, such as the southeastern section of the country, dry seasons are unpredictable. Planned irrigation of waste water from our lagoons was difficult. For example, during August 1977 our study area received over 35.6 cm of rain. This month is normally one of our drier months. Irrigation was out of the question. Manure spreading resulted in increased man hours, damage to pasture surface and drainage, and an increase in energy consumption. Stockpiling the manure during wet days resulted in odor and potential health problems. Five to ten cm rains are not uncommon in the southeast, and runoff simply removes nutrients before assimilation.

Regarding our system, in which we are interested in waste management (nutrient recovery and use), with an effective waste treatment process also being achieved, the nitrification-denitrification process posed a problem. Because inorganic nitrogen fertilizers require a high energy input to manufacture, maximum recovery of nitrogen from the lagoon system is desirable. Denitrification, which probably is most active at night, and in the lower portion of the lagoons, could result in a considerable loss of nitrogen as gas. Maintaining the system under complete anaerobic conditions and a pH of less than 10 or complete aerobic conditions should reduce the nitrogen loss and optimize the chance of recovery through the duckweed system. One particular advantage in using duckweeds is their capability of absorbing complex molecules (amino acids, sugars, etc.), thus having heterotrophic growth characteristics (Hillman, 1961). Not only can they remove complex organic nitrogen compounds, but the plants will continue to assimilate nutrients in the absence of light.

Through proper clonal selection strains of duckweeds may well be developed that greatly enhance nitrogen recovery. The addition of other nutrient-consuming organisms, with a marketable value, placed in a series of connecting lagoons could then provide for additional nutrient recovery and help offset or pay for the treatment facility. Figure 15 depicts such a


1 Two-acre lagoons
2 Skimmer
3 Harvesting canal
4 Washwater line
5 Conveyor/strainer
6 Washwater collection pit
7 Feed trailer
8 Feeding alley
9 Feeding trough
10 Feedlot covered with solar collector system
11 Duct drawing heated air from roof
12 Heat storage
13 Drying tunnel
14 Waste pit
15 Milking center
16 Fermentation unit
17 Gas scrubber
18 Methane gas storage
19 Generator
20 Waste sludge to lagoons via heat exchange in waste pit
21 Final stage lagoon treatment
22 Aquaculture system
23 Orchard
24 Pasture
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DRAWING BY DOUG REED
LSU COOPERATIVE EXTENSION SERVICE ART STUDIO

Figure 15. A schematic plan of an aquacultural waste treatment system involving duckweed.

schematic plan. In this system, emphasis is also given to energy recovery through the installation of a manure fermentation unit and solar collector system. Evidence presented in this study indicates that the proposed system shown in Figure 15 would be superior to land application.

## ANIMAL FEEDS

The "in vitro" digestibility of Wolffia sp. indicates a need for further study in culturing this plant. The clone was slow in adapting to lagoon conditions, but once established production appeared high. In preliminary trials for selecting the species for this study, a crude protein level of 30% was obtained for Wolffia. Under controlled nutrient systems the protein may approach other duckweeds.

The greatest disadvantage of Wolffia is its high water content, about 96%. Clonal selection should improve the condition however. If production and crude protein content approaches those of other duckweeds, then, due to its high digestibility more usable biomass will be realized. The high digestibility, indicating little fibrous tissue, places it as a potential human food.

# SECTION 9

# REFERENCES

American Chemical Society. 1969. Cleaning our environment: the chemical basis for action. Amer. Chem. Soc., Washington, D. C. 248 pp.

Anonymous. 1970. Agriculture poses waste problems. Envir. Sci. Technol. 4(12):1098-1100.

Anthony, W. B. 1969. Cattle Manure: Re-use through wastelage feeding. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 105-113.

Ashton, P. J. and R. D. Walmsley. 1976. The aquatic fern Azolla and its Anabaena symbiont. Endeavor, June (1976):39-43.

Bergey, E. H., R. S. Breed, and E. G. D. Murray. 1957. Bergey's manual of determinative bacteriology. 7th ed. Williams and Wilkens, Baltimore, Md.

Bhanthumnavin, K. and M. G. McGarry. 1971. Wolffia arrhiza as a possible source of inexpensive protein. Nature 232:495.

Buxton, B. M. and S. J. Zeigler. 1974. Economic impact of controlling surface water runoff from U.S. dairy farms. USDA Agr. Eco. Rep. No. 260.

Culley, D. D., Jr. and Ernest A. Epps. 1973. Use of duckweed for waste treatment and animal feed. J. Water Poll. Cont. Fed. 45:337-347.

Drigger, L. B., B. L. Carlile, S. H. Dobson, J. M. Falter, G. J. Kriz, J. L. Lutz, M. R. Overcash, G. S. Parson, J. A. Phillips, R. E. Sneed, and R. C. Wells. 1973. Dairy waste management alternatives. N.C. Agr. Expt. Serv. Bull. Circular 568.

Eby, Harry J. 1962. Manure lagoons - Design criteria and management. Agr. Engr. 43:698.

Foree, G. R. and R. A. O'Dell. 1969. Farm waste disposal field studies utilizing a modified pasveer oxidation ditch, settling tank, lagoon system. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 185-192.

Hamilton Standard. 1973. Draft development document for effluent limitations guidelines and standards of performance feedlot industry. Division of United Aircraft Corp., Windsor Locks, Conn. 275 pp.

Harvey, R. M. and J. L. Fox. 1973. Nutrient removal using *Lemna minor*. J. Water Poll. Contr. Fed. 45:1928-1938.

Henderson, Harry A. and L. L. Bauer. 1973. Cost of manure disposal on dairy farms in Tennessee. Univ. of Tenn. Agr. Expt. Sta. Bull. No. 514.

Hillman, W. S. 1957. Nonphotosynthetic light requirement in *L. minor* and itspartial satisfaction by Kinetin. Science 126:165-166.

Hillman, W. S. 1961. The Lemnaceae, or duckweeds. A review of the descriptive and experimental literature. Bot. Rev. 27:221-289.

Hogland, C. R., J. S. Boyd, L. J. Connor, and J. B. Johnson. 1972. Waste management practices and systems on Michigan dairy farms. Michigan State Agr. Eco. Rep. No. 208.

Horwitz, W. (ed.). 1970. Methods of analysis of the association of official analytical chemists. Assoc. Off. Anal. Chemists. Washington, D. C. 1015 pp.

Hromadka, M. P. 1976. A cost comparison of selected waste handling systems for Louisiana dairy farms. Unpublished Masters Thesis, Dept. of Ag. Economics, La. State Univ., Baton Rouge.

Joy, K. W. 1969. Nitrogen metabolism of *Lemna minor*. I. Growth nitrogen sources and amino acid inhibition. Plant Physiol. 44:845-848.

Just, R. E., T. R. Nelson, W. L. Brant, and D. R. Gill. 1969. Feedmix program. As adapted by C. W. Pope for La. conditions (computer software), La. Coop. Ext. Serv., La. State Univ., Baton Rouge.

Loehr, R. C. 1969. Animal wastes--a national problem. J. Sanit. Eng. Div. Proc. Am. Soc. Civ. Engr. SA2:189.

Loehr, R. C. 1977. Pollution control for agriculture. Academic Press, Inc., New York, N.Y.

McCaskey, T. A., J. A. Little, and G. H. Rollins. 1971. Methods of handling bovine waste and a study of optimum rates for land disposal. Presented at Meeting of Assoc. of Southern Agr. Workers, Inc. Jacksonville, Florida.

Miner, J. R. 1975. Farms animal - waste management. Iowa Agr. Expt. Sta. Spec. Rep. 67.

National Academy of Sciences. 1971. Atlas of nutritional data on United States and Canadian feeds. Washington, D. C. 772 pp.

National Academy of Sciences. 1976. Making aquatic weeds useful: some perspectives for developing countries. Washington, D. C. 175 pp.

Overman, A. R., C. C. Hortenstine, and J. M. Wing. 1970. Land disposal of dairy farm wastes. In Relationship of Agriculture to Soil and Water Pollution. Proceedings Cornell University Conference on Agricultural Waste Management. 1969, p. 123-126.

Painter, H. A. 1970. A review of the literature on inorganic nitrogen metabolism in microorganisms. Water Res. 4:393.

Patrick, W. H., R. D. Delaune, R. M. Engler and S. Gotoh. 1976. Nitrate removal from water at the water-mud interface in wetlands. EPA-600/3-76-042. U.S. Environmental Protection Agency, Corvallis, Oregon. 80 pp.

Russell, E. W. 1961. Soil conditions and plant growth. p. 316 (9th ed.). Wiley and Sons. New York, N.Y. 688 pp.

Samples, W. R. 1967. Removal of nitrogenous compounds from wastewaters. Chem. Eng. Prog. Symp. Ser., 63:78.

Schmid, L. A. and R. I. Lipper. 1969. Swine wastes, characterization and anaerobic digestion. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 50-57.

Scotland, M. B. 1934. The animals of the *Lemna* association. Ecology 15(3):290-294.

Sobel, A. T. 1969. Removal of water from animal manures. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 347-362.

Society of American Bacteriologists. 1957. Manual of microbiological methods. McGraw-Hill Book Co., New York, N.Y.

Stanley, Ronald A. and Carl E. Madewell. 1975. Optimum dilution of swine wastes for growth of *Lemna minor* L. and Euglena sp. In Managing Livestock Wastes. Amer. Soc. Agr. Engr., p. 331-333.

Sutton, D. L. and W. H. Ornes. 1975. Phosphorus removal from static sewage effluent using duckweed. J. Environ. Qual. 4:367-370.

Truax, R. E., D. D. Culley, Jr., Melvin Griffith, W. A. Johnson, and J. P. Wood. 1972. Duckweed for chick feed? Louisiana Agr. 16(1):8-9.

Turner, G. 1970. An agriculture engineer's opinion about lagoons. J. Milk and Food Tech. 33(11):497.

van Fassen, H. G. and H. van Dijk. 1975. Oxidation-nitrification and denitrification of veal calf manure. In Managing Livestock Wastes. Amer. Soc. Agr. Engr., p. 314-317.

Vickers, A. F. and E. J. Genetelle. 1969. Design parameters for the stabilization of highly organic manure slurries by aeration. Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 37-49.

Wells, W. N. 1969. Differences in phosphate uptake rates exhibited by activated sludges. J. Water Pollut. Contr. Fed. 41:765-771.

Wheatland, A. B., M. J. Barrett, and A. M. Bruce. 1959. Some observations on denitrification in rivers and estuaries. Proc. J. Inst. Sewage Purif. 2:258-271.

White, J. H. and F. J. Holben. 1921. Soil fertility experiments on DeKalb, Voluria, and Westmoreland soils. Penn. State Col. Agr. Expt. Sta. Bull. 166. 23 pp.

Wiancke, A. T., G. P. Walker, and R. R. Mulvey. 1935. Manure increases farm income. Purdue Univ. Agr. Expt. Sta. Bull. 398. 20 pp.

Wolverton, B. C., R. M. Barlow, and R. C. McDonald. 1976. Application of vascular aquatic plants for pollution removal, energy, and food production in a biological system. NASA Tech. Memo. TM-X-72726.

# SECTION 10

## BIBLIOGRAPHY

Alich, J. A. and R. E. Inman. 1973. Effective utilization of solar energy to produce clean fuel. In Bioconversion, Hearing Before the Subcommittee on Energy, Committee on Science and Astronautics. U.S. House of Representatives Ninety-third Congress (No. 40). U.S. Govt. Printing Office, Washington, D. C.

Allen, G. H. and L. Dennis. 1974. Report on pilot aquaculture systems using domestic wastewaters for rearing Pacific salmon smolts. In Wastewater Use in the Production of Food and Fiber, Proceedings, EPA-660/2-74-041. U.S. Environmental Protection Agency. Washington, D. C. p. 162-198.

Allen, J. B. and B. Lowrey. 1976. Methane gas production from dairy poultry and swine anaerobic lagoons. Southeast Am. Soc. of Agr. Engr., Mobile, Al., Feb. 1-4, 1976.

Allen, J. B. and J. C. McWhorter. 1971. Field crop irrigation with oxidation pond effluent. 1971 Annual Meeting Amer. Soc. Agr. Engr. 29 pp.

Bandel, V. A., S. Dzienia, G. Stanford and J. O. Legg. 1975. N behavior under no-till vs. conventional corn culture. I. First-year results using unlabeled N fertilizer. Agron. J. 67:782-786.

Bhagat, S. K. and D. E. Proctor. 1969. Treatment of Dairy Manure by Lagooning. J. Water Pollut. Contr. Fed. 41:785-795.

Blackburn, R. D. and D. L. Sutton. 1971. Growth of the white amur (Ctenopharyngodon idella Val.) on selected species of aquatic plants. Proc. Eur. Weed Res. Coun., 3rd Int. Symp. Aquatic Weeds. p. 87-93.

Bouwer, H. 1968. Returning wastes to the land, a new role for agriculture. J. Soil and Water Cons. 23:164.

Boyd, C. E. 1968. Some aspects of aquatic plant ecology. In Symposium on Reservoir Fishery Resources. University of Georgia Press, Athens. p. 114-129.

Boyd, C. E. 1968. Evaluation of some common aquatic weeds as possible feedstuffs. Hyacinth Cont. J. 7:26-27.

Boyd, C. E. 1968. Fresh-water plants: a potential source of protein. Econ. Bot. 22:359-368.

Boyd, C. E. 1969. The nutritive value of three species of water weeds. Econ. Bot. 23:123-127.

Boyd, C. E. 1970. Vascular aquatic plants for mineral nutrient removal from polluted waters. Econ. Bot. 24:95-103.

Boyd, C. E. 1970. Amino acid, protein, and caloric content of vascular aquatic macrophytes. Ecology 51:902-906.

Bryan, F. L. 1974. Diseases transmitted by foods contaminated by wastewater. In Wastewater Use in the Production of Food and Fiber, Proceedings, EPA-660/2-74-041. U.S. Environmental Protection Agency. Washington, D. C. p. 16-45.

Carpenter, R. L., M. S. Coleman, and R. Jarman. 1976. Aquaculture as an alternative wastewater treatment system. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 215-224.

Chang, A. C., A. C. Dale, and M. Bell. 1971. Nitrogen transformations during aerobic digestion and denitrification of dairy cattle wastes. In Livestock Waste Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 272-274.

Chang, A. C. and J. M. Rible. 1975. Particle size distribution of livestock wastes. In Managing Livestock Wastes. Amer. Soc. Agr. Engr., p. 339-342.

Chomchalow, N. 1971. Miracle Plant. Agricultural Science (Thai Agric. Sci. Assoc., Bangkok) 4(4):319-330.

Clark, C. E. 1965. Hog waste disposal by lagooning. J. Sanit. Engr. Div., Amer. Soc. Civil Engr. 91, SA6, p. 27-42.

Czerwenka, W. and K. Seidel. 1976. The combination of biological and chemical treatment at the Krefeld water treatment works. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 287-293.

Dale, A. C., J. R. Ogilvie, A. C. Chang, M. P. Douglass, and J. A. Lindley. 1969. Disposal of dairy cattle wastes by aerated lagoons and irrigation. Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 150-159.

Daubs, E. H. 1965. A monograph of Lemnaceae. Illinois Biological Monograph No. 34. Univ. of Illinois Press, Urbana. 118 pp.

Dawson, R. N. and K. L. Murphy. 1972. The temperature dependency of biological denitrification. Water Res. 6:71-83.

De Lange, L. 1975. Gibbosity in the complex Lemna gibba/Lemna minor: literature survey and ecological aspects. Aquat. Bot. 1:327-332.

Dinges, R. S. 1976. A proposed integrated biologist wastewater treatment system. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 225-230.

Dugan, G. L., C. G. Golueke, and W. J. Oswald. 1972. A recycling system for poultry wastes. J. Water Pollut. Contr. Fed. 44(3):432-440.

Dunn, G. G. and J. B. Robinson. 1972. Nitrogen losses through denitrification and other changes in continuously aerated poultry manure. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1972, p. 545-554.

Edwards, J. B. and J. B. Robinson. 1969. Changes in composition of continuously aerated poultry manure with species reference to nitrogen. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 78-184.

Ehrlich, S. 1966. Two experiments in the biological clarification of stabilization-pond effluents. Hydrobiologia 27:70-80.

Environmental Protection Agency. 1972. Cattle feedlots and the environment: control guidelines. U.S. Environmental Protection Agency, Seattle, Washington.

Environmental Protection Agency. 1975. Process design manual for phosphorus control. EPA625/1-76-001a, U.S. Environmental Protection Agency, Washington, D. C.

Foster, D. H. and R. S. Engelbrecht. 1973. Microbial hazards in disposing of wastewater on soil. In Recycling Treated Municipal Wastewater and Sludge Through Forest and Cropland, eds. W. E. Sopper and L. T. Kardos, p. 247-270.

Goldman, J. C. and J. H. Rhyther. 1976. Waste reclamation in an integrated food chain system. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 197-214.

Golueke, C. G. 1974. Biological reactions in waste recovery systems. Compost Sci. 15(3):2-6.

Gorham, P. R. 1964. Toxic algae as a public health hazard. J. Am. Water Works Assoc. 56(11):1481.

Greenland, D. J. 1975. Bringing the green revolution to the shifting cultivator. Science 190:841-844.

Haller, W. T., D. L. Sutton, and W. C. Barlowe. 1974. Effects of salinity on growth of several aquatic macrophytes. Ecology 55:891-894.

Hart, S. A. and M. E. Turner. 1965. Lagoons for livestock manure. J. Water Pollut. Contr. Fed. 37:1578-1596.

Hegg, R. O. and E. R. Allred. 1975. Nitrogen transformation in aerated beef slurries. In Managing Livestock Wastes. Amer. Soc. Agr. Engr., p. 514-521.

Hendricks, D. W. and W. D. Pote. 1974. Thermodynamic analysis of a primary oxidation pond. J. Water Pollut. Contr. Fed. 46(2):333-352.

Hillman, W. S. 1975. Photoperiodism in seedling strains of Lemna perpusilla: juvenility without obvious morphological correlates? Amer. J. Bot. 62:537-540.

Hillman, W. S. 1976. Calibrating duckweeds: light, clocks, metabolism, flowering. Science 193:453-458.

Hunt, P. G. and C. R. Lee. 1976. Land treatment of wastewater by overland flow for improved water quality. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Phildelphia, Pa. p. 151-160.

Hutchinson, G. E. 1975. Treatise on limnology. Vol. 3: Limnological Botany. Wiley and Sons, New York, N.Y.

Jewell, W. J. 1975. Energy, agriculture and waste management. Ann Arbor Science Publ., Ann Arbor, Mich.

Jones, P. H. and N. K. Patni. 1974. Nutrient transformations in a swine waste oxidation ditch. J. Water Pollut. Contr. Fed. 46:366-379.

Katzenelson, E., I. Buium, and H. I. Shuval. 1976. Risk of communicable disease associated with wastewater irrigation on agricultural settlements. Science 194:944-946.

Kemp, C. C. and G. C. Szego. 1974. The energy plantation. In Bioconversion. Hearing Before the Subcommittee on Energy, Committee on Science and Astronautics, U.S. House of Representatives, Ninety-third Congress, Second Session (No. 40). U.S. Govt. Printing Office, Washington, D. C.

King, D. L. 1972. Carbon limitation in sewage lagoons. In Nutrients and Eutrophication. G. E. Liken, ed. Amer. Soc. Lim. and Ocean. 1:98-105.

Landolt, E. 1957. Physiologische und ökologische untersuchungen and Lemnaceen. Ber. schweiz. bot. Ges. 67:271-410.

Lawson, T. B., H. J. Braud, and F. T. Wratten. 1974. Methods of drying duckweed, Lemnaceae. Amer. Soc. Agr. Engr., 1974 Winter Meeting, Chicago, Ill.

Lewis, W. M. and M. Bender. 1961. Effect of a cover of duckweeds and the alga Pithophora upon the dissolved oxygen and free carbon dioxide of small ponds. Ecology 42:602-603.

Loehr, R. C. 1968. Characteristics of farm effluents. In Handbook of Environmental Control. Chemical Rubber Company, 4:637.

Loehr, R. C. 1969. Treatment of wastes from beef cattle feedlots - field results. In Proceedings Animal Waste Management Cornell University Conference on Agricultural Waste Management, 1969, p. 225-241.

Loehr, R. C., D. R. Anderson, and A. C. Anthonisen. 1971. An oxidation ditch for the handling and treatment of poultry wastes. In Livestock Waste Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 209-212.

Loehr, R. C. 1974. Agricultural waste management. Academic Press, New York, N.Y. 576 pp.

Lorimor, J. C., S. W. Melvin, and B. M. Leu. 1975. Nutrient characteristics of wastes from deep pits and anaerobic lagoons. In Managing Livestock Wastes. Amer. Soc. Agr. Engr., p. 306-308.

Mathers, A. C. and B. A. Stewart. 1971. Crop production and soil analyses as affected by application of feedlot waste. In Livestock Waste Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 229-231.

McKinney, R. E. 1970. Manure transformations and fate of decomposition products in water. In Agricultural Practices and Water Quality, eds. T. D. Willrich and G. E. Smith. Iowa St. Univ. Press, Ames. p. 256-264.

McLay, C. L. 1976. The effect of pH on the population growth of thre species of duckweed: Spirodela oligorhiza, Lemna minor and Wolffia arrhiza. Freshwater Biology 6:125-136.

Miller, V. 1977. Metal-roofed shed is solar heat collector. Progressive Farmer, April 1977:48.

Miner, J. R., J. W. Wooten, and J. D. Dodd. 1971. Water hyacinths to further treat anaerobic lagoon effluent. In Livestock Waste Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 170-173.

Miner, J. R. and R. J. Smith. 1975. Livestock waste management with pollution control. Agricultural Experiment Station, North Central Regional Research Publication 222. Midwest Plan Service Handbook NWPS-19, Iowa State Univ., Ames. 89 pp.

Nichols, S. A. 1974. Mechanical and habitat manipulation for aquatic plant management. Technical bulletin No. 77. Dept. Natural Resources, Madison, Wis. 34 pp.

Pieterse, A. H. 1976. Physiological, morphological and anatomical aspects of gibbosity in Lemna gibba. Aquat. Bot. 1:333-344.

Pirie, N. W. 1966. The merits of food proteins from novel sources. Sci. Progress. 54:401-412.

Porter, K. G. 1977. The plant-animal interface in freshwater ecosystems. Amer. Scientist 65:159-170.

Posner, H. B. and W. S. Hillman. 1962. Aseptic production, collection and germination of seeds of Lemna perpusilla 6746. Physiol. Plantarum 15:700-708.

Rappaport, R. A. 1971. The flow of energy in an agricultural society. Scient. Amer. 225(3):117-132.

Rejmankova, E. 1975. Comparison of Lemna gibba and Lemna minor from the production ecological viewpoint. Aquat. Bat. 1:423-427.

Rodgers, H. H. and D. E. Davis. 1972. Nutrient removal by water hyacinth. Weed Sci. 20:423-428.

Rowe, M. L. and L. Merryman. 1977. Livestock and the environment: a bibliography with abstracts - Vol. IV. EPA-600/2-77-092. U.S. Environmental Protection Agency, Ada, Ok. 569 pp.

Seidel, K. 1976. Macrophytes and water purification. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 109-121.

Silveston, P. L. 1975. Methane production from manure in small scale units. In Symposium on Energy from Biological Conversion. 79th National Meeting Amer. Institute of Chem. Engrs. Houston, Tx. Paper No. 9.

Skerman, V. B. D. and I. C. MacRae. 1957. Influence of oxygen availability on the degree of nitrate reduction by Pseudomonas denitrificans. Can. J. Microbiol. 3:505-530.

Smith, R. J., T. E. Hazen, and J. R. Miner. 1971. Manure management in a 700 head swine-finishing building: two approaches using renovated wastewater. In Livestock Waste Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 149-153.

Spangler, F., W. Sloey, and C. W. Fetter. 1976. Experimental use of emergent vegetation for the biological treatment of municipal wastewater in Wisconsin. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 161-171.

Standifer, L. C. and M. N. Ismail. 1975. A multiple cropping system for vegetable production under subtropical high rainfall conditions. J. Amer. Soc. Hort. Sci. 100(5):503-506.

Stanely, R. A. 1974. Methods of biological recycling of nutrients from livestock waste: a literature review and systems analysis. Bulletin Y-80, Nat. Fertilizer Dev. Center, Tennessee Valley Authority, Muscle Shoals, Al.

Stanley, R. A. and C. E. Madewell. 1976. Chemical tolerance of Lemna minor, L. Tennessee Valley Authority, Muscle Shoals, Al. 17 pp.

Stanley, R. A. and C. E. Madewell. 1976. Thermal tolerance of Lemna minor, L. Tennessee Valley Authority, Muscle Shoals, Al. 16 pp.

Stewart, T. A. and R. McIlwain. 1971. Aerobic storage of poultry manure. In Livestock Management and Pollution Abatement. Amer. Soc. Agr. Engr., p. 261-262.

Strauss, R. 1976. Effet de divers sels alcalins sur la croissance et nutrition minérale de Lemna minor L. Int. Rev. ges Hydrobiol. 61:673-676.

Tourbier, J. and R. W. Pierson, Jr., eds. 1976. Biological Control of Pollution, University of Pennsylvania Press, Philadelphia, Pa. 240 pp.

Ward, R. F. 1974. Renewable fuels. In Bioconversion. Hearing Before the Subcommittee on Energy, Committe on Science and Astronautics, U.S. House of Representatives Ninety-third Congress (No. 40). U.S. Govt. Printing Office, Washington, D. C.

Westlake, D. F. 1963. Comparisons of plant productivity. Biol. Review 38:385.

Wetzel, R. G. 1972. The role of carbon in hard-water marl lakes. In G. E. Likens, ed., Nutrients and Eutrophication: The limiting nutrient controversy. Spec. Symp., Amer. Soc. Limnol., Oceanogr., 1:84-91.

Wolf, M. 1974. Utilization of solar energy by bioconversion--an overview. In Bioconversion. Hearing Before the Subcommittee on Energy, Committee on Science and Astronautics, U.S. House of Representatives (No. 40). p. 2-33.

Wolverton, B. C., R. M. Barlow, and R. C. McDonald. 1976. Application of vascular aquatic plants for pollution removal, energy and food production in a biological system. In Biological Control of Water Pollution, eds. J. Tourbier and R. W. Pierson. University of Pennsylvania Press, Philadelphia, Pa. p. 141-149.

Woodwell, G. M., J. T. Ballard, J. Clinton, and E. V. Pecan. 1976. Nutrients, toxins and water in terrestrial and aquatic ecosystems treated with sewage plant effluents. Final Report of the Upland Recharge Program. (BNL 50513; Biological and Medicine TID-4500). Brookhaven National Laboratory, Upton, N.Y. 11973. 39 pp.

Yount, J. L. and R. A. Crossman, Jr. 1970. Eutrophication control by plant harvesting. J. Water Pollut. Contr. Fed. 42, 173.